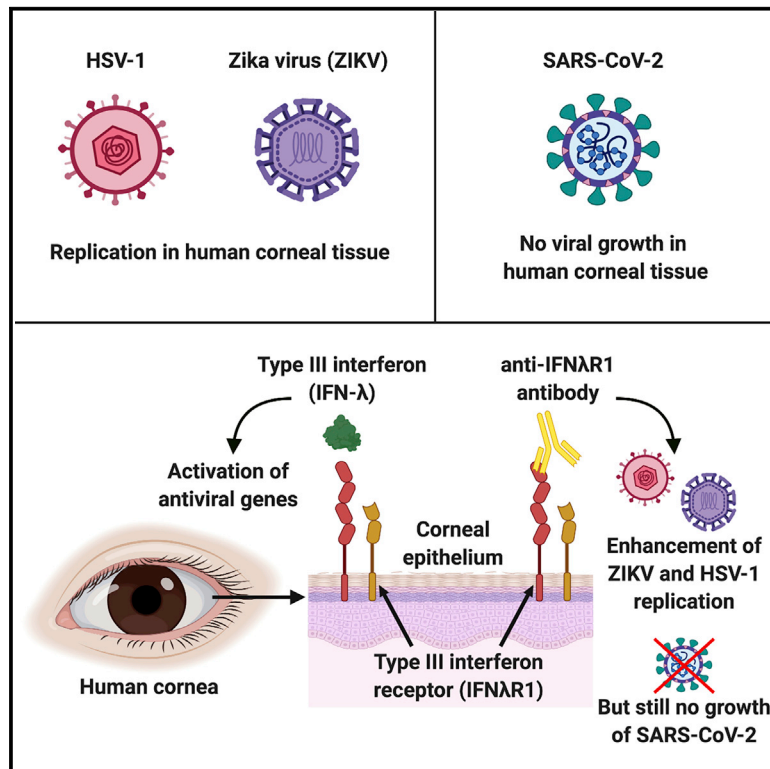


HSV-1 and Zika Virus but Not SARS-CoV-2 Replicate in the Human Cornea and Are Restricted by Corneal Type III Interferon

Graphical Abstract



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In Brief

Miner et al. report that type III interferon (IFN- λ) and its receptor (IFN λ R1) restrict herpes simplex virus 1 (HSV-1) and Zika virus (ZIKV) growth in the human cornea. Human corneal explants did not support severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, even after blockade of the type III IFN receptor.

Highlights

- The type III interferon receptor (IFN λ R1) inhibits viral growth in the human cornea
- Zika virus is transmissible by corneal transplantation in mice
- IFN- λ limits herpes simplex virus 1 and Zika virus growth in the human cornea
- SARS-CoV-2 does not replicate in human corneal tissue



Report

HSV-1 and Zika Virus but Not SARS-CoV-2 Replicate in the Human Cornea and Are Restricted by Corneal Type III Interferon

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SUMMARY

Here, we report our studies of immune-mediated regulation of Zika virus (ZIKV), herpes simplex virus 1 (HSV-1), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the human cornea. We find that ZIKV can be transmitted via corneal transplantation in mice. However, in human corneal explants, we report that ZIKV does not replicate efficiently and that SARS-CoV-2 does not replicate at all. Additionally, we demonstrate that type III interferon (IFN- λ) and its receptor (IFN λ R1) are expressed in the corneal epithelium. Treatment of human corneal explants with IFN- λ , and treatment of mice with IFN- λ eye drops, upregulates antiviral interferon-stimulated genes. In human corneal explants, blockade of IFN λ R1 enhances replication of ZIKV and HSV-1 but not SARS-CoV-2. In addition to an antiviral role for IFN λ R1 in the cornea, our results suggest that the human cornea does not support SARS-CoV-2 infection despite expression of ACE2, a SARS-CoV-2 receptor, in the human corneal epithelium.

INTRODUCTION

The Zika virus (ZIKV) epidemic and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic have led to speculation that these viruses may, in some cases, enter the body through the eyes. Other viruses, including herpes simplex virus 1 (HSV-1), are well known to efficiently replicate in the cornea. Indeed, corneal infection with HSV-1 is a major cause of corneal damage and vision loss (Farooq and Shukla, 2012). However, the immune mechanisms that regulate infection of the human cornea are incompletely understood.

During viral infections, type I and type III interferons (IFNs) induce expression of IFN-stimulated genes (ISGs) that restrict viral replication and spread (Hoffmann et al., 2015; Lazear et al., 2015; Miner et al., 2016a, 2016b). Whereas the type I IFN receptor (IFNAR1) is expressed ubiquitously, the type III IFN receptor (IFN λ R1; IL28RA) has a more restricted expression pattern, leading to antiviral effects that are especially notable at barrier surfaces, including the placenta, gut, urogenital tract, and blood-brain barrier (Bayer et al., 2016; Jagger et al., 2017; Lazear et al., 2015; Nice et al., 2015). A role for IFN λ R1 signaling in the human cornea has not previously been described.

HSV-1 infection of the human cornea requires emergency treatment, including antiviral and corticosteroid therapy to

help prevent corneal opacification and to preserve vision (Farooq and Shukla, 2012). HSV-1 keratitis alone is responsible for ~40,000 new cases of visual impairment and blindness globally each year (Farooq and Shukla, 2012). Other viruses, including flaviviruses (e.g., ZIKV) also trigger ocular inflammation (e.g., conjunctivitis, uveitis) and can be detected in the vitreous humor in rare cases (de Paula Freitas et al., 2017; Heck et al., 2017; Kodati et al., 2017; Miner et al., 2016b). Additionally, SARS-CoV-2 RNA has been detected in tears (Fang et al., 2020; Karimi et al., 2020; Wu et al., 2020), and it has been widely hypothesized that the eye, including the cornea and conjunctiva, may serve as a route of entry for SARS-CoV-2.

ZIKV does not replicate efficiently in immunocompetent mice (Grant et al., 2016). Therefore, in our prior studies of ZIKV pathogenesis in the eye, it was necessary to study immunodeficient *Irfar1*^{-/-} animals (Miner et al., 2016b). We found that ZIKV invades and replicates in the eyes of mice and that ZIKV RNA was detectable in the cornea. However, we did not test whether viral RNA found in the cornea was associated with infectious virus (Miner et al., 2016b). Subsequently, humans with ZIKV infection were reported to have ZIKV RNA and infectious ZIKV in conjunctival swabs (Sun et al., 2017). ZIKV-induced uveitis and conjunctivitis also occur in humans (de Paula Freitas et al.,



2017; Kodati et al., 2017), suggesting that ZIKV can infect the eye and cause ocular inflammation.

Here, we report that HSV-1 and ZIKV, but not SARS-CoV-2, can replicate in human corneal explants. We found that type III IFN (also known as IFN- λ) and its receptor (IFN λ R1) are expressed and functional in the human and mouse cornea. Using a model of corneal transplantation in mice, we found that ZIKV is only rarely transmitted by corneal transplantation in immunodeficient AG129 mice that lack the type I and type II IFN receptors. This led us to hypothesize that IFN- λ may play a role in controlling infection in the cornea. Indeed, we found that IFN- λ and IFN λ R1 are expressed within and exhibit antiviral activity in cultured human corneal explants. Furthermore, we found that antiviral ISGs are constitutively upregulated in the epithelium of human corneal explants and that treatment of mice with IFN- λ eye drops causes IFN λ R1-dependent upregulation of antiviral ISGs. Blockade of IFN λ R1 in human corneal explants led to enhanced replication of ZIKV and a \sim 10-fold increase in replication of HSV-1. In contrast, blockade of IFN λ R1 did not result in permissiveness of human corneal tissue to infection with SARS-CoV-2. Thus, IFN λ R1 restricts HSV-1 and ZIKV infection in human corneal tissue. Furthermore, resistance of human corneal tissue to SARS-CoV-2 infection is likely regulated by a distinct antiviral pathway.

RESULTS AND DISCUSSION

ZIKV Transmission by Corneal Transplantation in Mice

Although we previously detected ZIKV RNA in mouse corneas after subcutaneous inoculation, we did not test whether this viral RNA represents infectious viral particles (Miner et al., 2016b). AG129 mice, which lack both type I and type II IFN receptors (van den Broek et al., 1995), are highly susceptible to ZIKV. Indeed, even a single infectious particle is sufficient to cause death in AG129 animals (Aliota et al., 2016). To test whether infectious virus was present in mouse corneas following subcutaneous inoculation with ZIKV, we intraperitoneally inoculated 8-week-old AG129 animals with corneal homogenates after microdissection of eyes from *Ifnr1*^{-/-} mice 7 days after subcutaneous inoculation with a clinical ZIKV isolate from Brazil. Three of 11 AG129 mice inoculated with corneal homogenates developed ZIKV encephalitis and succumbed to infection (Figure 1A). Thus, infectious ZIKV is associated with the corneas of *Ifnr1*^{-/-} mice following subcutaneous footpad inoculation.

Transmission of viruses by solid organ transplantation is a major clinical concern that requires careful safeguards (Heck et al., 2017; Nogueira et al., 2017). Furthermore, ZIKV transmission via solid organ transplantation has been previously described in humans (Nogueira et al., 2017). To test whether ZIKV transmission might be possible after corneal transplantation in animals, we performed corneal transplantation experiments in AG129 mice. Donor AG129 mice were subcutaneously inoculated with 1,000 FFU of ZIKV and euthanized 7 days after infection, followed by tissue harvesting and microdissection of corneas. We confirmed the presence of 10⁵–10⁶ FFU equivalents of ZIKV in the posterior eye, liver, spleen, brain, kidney, and serum of infected donor animals (Figure 1B). The donor corneas were washed extensively in PBS and subsequently transplanted into naive AG129 recipient

mice. Six days after transplantation, recipient mice were euthanized, and ZIKV RNA in multiple organs was quantitated using qRT-PCR. Although viral RNA was detectable in multiple organs at low levels in all recipient animals, severe infection of multiple organs occurred in just one of the six recipient mice (Figure 1C). Thus, ZIKV can cause systemic infection following corneal transplantation in mice, but this occurs infrequently. Next, to evaluate whether ZIKV remains stable and infectious in cornea storage medium, we tested the stability of ZIKV in Optisol. ZIKV (6 × 10⁵ FFU per tube) was stored in Optisol at 4°C for 1 month, followed by quantitation of infectious virus by focus forming assay. By day 28, more than 10³ FFU/mL of infectious ZIKV was still present under these conditions (Figure 1D), indicating that infectious ZIKV can persist in conditions typical for storage of human donor corneas.

ZIKV Infects the Human Corneal Epithelium but Does Not Replicate Efficiently in Human Corneal Explants

To test whether ZIKV replicates in human corneal tissue, we performed viral growth curve studies by inoculating human corneal explants with 3 × 10⁶ FFU/mL of ZIKV, followed by focus forming assays over the course of 72 h (Figures 1E and F). ZIKV replicated inefficiently in just one of three human cornea donor samples (Figure 1E), suggesting that the human cornea is somewhat resistant to ZIKV infection. To define ZIKV tropism in the human cornea, we performed RNA *in situ* hybridization (ISH) of ZIKV-infected corneal explants 24 h after infection (Figure 1F). Histological analysis revealed that ZIKV preferentially infects a small number of corneal epithelial cells, without appreciably penetrating into deeper layers of the human cornea. We hypothesized that constitutive IFN signaling may be controlling viral infection in the human corneal explants, so we measured IFN and ISG expression in human corneal explants cultured in the absence of virus. We observed expression of type III IFNs (*IFNL1*, *IFNL2*) and *IFIT1* (an ISG) mRNA, even in the absence of infection (Figure 1G). As *IFNL1* and *IFNL2* expression is regulated by *IRF3*, we hypothesized that either cytosolic DNA- or RNA-sensing pathways may upregulate *IFNL1* or *IFNL2* via *IRF3* downstream of TBK1 signaling induced by endogenous retroelements. To test this hypothesis, we treated corneas with an antiretroviral drug (AZT) or TBK1 inhibitor (MRT67307), but neither of these inhibitors affected basal expression of *IFNL1* or *IFNL2* (Figure S1), suggesting that basal expression of *IFNL1* and *IFNL2* are regulated independently of TBK1-mediated phosphorylation of IRF3.

Treatment of Mouse Corneal Explants with IFN- λ Upregulates Expression of ISGs

As IFN- λ restricts infection at some barrier surfaces, we reasoned that topical treatment with IFN- λ eye drops might upregulate ISG expression in mice specifically in the cornea. To test this hypothesis, we administered two doses of 1 μ g topical recombinant IFN- λ eye drops or PBS control to wild-type (WT) and *Ifnr1*^{-/-} mice, followed by microdissection of corneas and quantitation of ISG expression using qRT-PCR. In WT mice, but not *Ifnr1*^{-/-} mice, treatment with IFN- λ caused upregulation of multiple ISGs, including *Iffit2*, *Iffit3*, and *Isg15*, but not *Iffit27* (Figures 2A–2E). These results indicate that topical IFN- λ induces

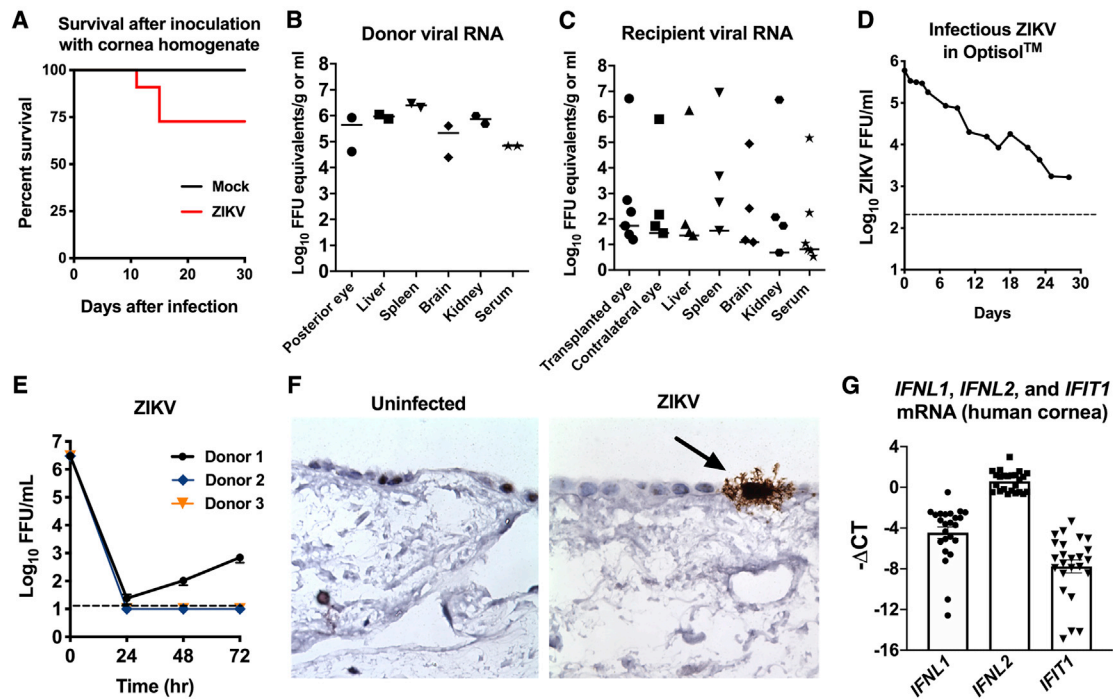


Figure 1. ZIKV Can Be Transmitted via Corneal Transplantation in AG129 Mice but Infects Human Corneal Explants Inefficiently

(A) Survival of AG129 mice inoculated with *Ifnar1*^{-/-} corneal homogenates 7 days after subcutaneous inoculation with ZIKV (n = 11) or uninfected *Ifnar1*^{-/-} cornea control homogenates (n = 4).
 (B and C) ZIKV mRNA expression in tissues was quantitated using qRT-PCR. Tissues were harvested from cornea donors (n = 2) and recipient (n = 6) mice on days 6 and 7 after infection.
 (D) ZIKV (6×10^5 FFU) was stored in cornea storage medium (Optisol) at 4°C for 28 days. Infectious virus was measured by focus forming assay at the indicated time points.
 (E) Human donor corneas (n = 4 technical replicates per donor from three independent donor corneas) were inoculated with 3×10^6 FFU/mL of ZIKV (Brazil strain), and infectious virus was quantitated at the indicated time points by focus forming assay. Data represent the mean \pm SEM.
 (F) RNA ISH and histological analysis was performed on uninfected (left panel) and ZIKV-infected (right panel) human corneal explants in order to determine virus tropism. The black arrow indicates infected corneal epithelial cells.
 (G) Baseline expression of *IFNL1*, *IFNL2*, and *IFIT1* was measured using qRT-PCR in three human donor corneas in independent experiments. Data represent the mean Δ CT \pm SEM relative to expression of GAPDH. Each cornea was sectioned to produce eight technical replicates per experiment (n = 24) from three biological replicates in independent experiments.

antiviral gene expression in the mouse cornea under physiological conditions.

To determine whether IFN- λ promotes antiviral immunity in the human cornea, we began by performing RNA ISH and discovered that IFN λ R1 is expressed specifically in the human corneal epithelium (Figure 3A), which are target cells for viruses known to cause corneal pathology, including HSV-1 (Oh, 1976). Treatment of human corneal explants with 4×10^4 U/mL of IFN- λ for 6 h led to a \sim 2-fold increase in *IFNLR1* mRNA (Figure 3B), as well as a \sim 50-fold increase in expression of *IFIT1* and a \sim 25-fold increase in *IFIT2* (Figures 3C and 3D). *IFI27* and *IRF7* also were upregulated by approximately \sim 3-fold to 5-fold after treatment with IFN- λ (Figures 3E and 3F). Thus, IFN- λ induces expression of antiviral ISGs in the human cornea.

To test whether IFN- λ protects against infection with ZIKV, we pre-treated human donor corneas for 1 h with either anti-IFN λ R1 monoclonal antibody (mAb) or a control mAb (Kotenko et al., 2003) and then performed viral growth curve analysis as described in Figure 1. In two of five samples, blockade of the

IFN λ R1 resulted in an approximately 100-fold greater ZIKV replication (Figure 3G). However, in other growth curve experiments of ZIKV in the human cornea, viral replication was not detected (data not shown). This suggests that the role of IFN λ R1 in controlling ZIKV infection is variable in distinct donors.

Next, as HSV-1 is well established as a corneal pathogen in humans, we inoculated human corneal explants with 10^6 PFU/mL of HSV-1 in the presence of the same blocking anti-IFN λ R1 mAb or control mAb (Kotenko et al., 2003). This led to consistently enhanced replication of HSV-1 in all infected human donor samples, with approximately 10-fold higher levels of infectious virus by 72 h after infection (6.1×10^4 PFU/mL in anti-IFN λ R1 treated corneas versus 6.3×10^3 PFU/mL in control mAb-treated corneas, $p < 0.005$) (Figure 3H). HSV-1 blocks the type I IFN response in order to facilitate viral replication (Yuan et al., 2018), so we reasoned that HSV-1 may similarly antagonize IFN- λ production or signaling. Quantitation of *IFNL1* and *IFNL2* mRNA with and without HSV-1 revealed that HSV-1 infection reduces expression of *IFNL2* within the first 24 h after infection

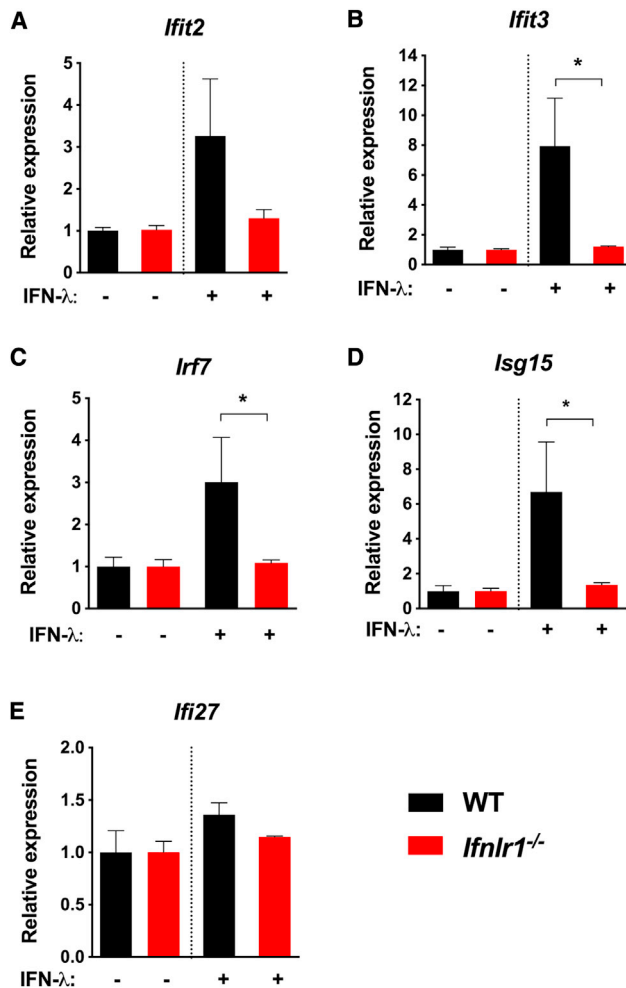


Figure 2. IFN- λ Eye Drops Induce ISG Expression in WT but Not *Ifnlr1*^{-/-} Corneas

Adult WT and *Ifnlr1*^{-/-} animals were treated with recombinant mouse IFN- λ (1 μ g) in 10 μ L PBS or PBS vehicle control. Mice were treated again 3 h later and then euthanized for corneal microdissection 6 h after the first treatment. Corneas were homogenized in PBS, and mRNA was isolated for gene expression analysis. ISG expression in corneas was quantitated using qRT-PCR (n = 3–5 mice per group). Results represent the mean \pm SEM of data pooled from two independent experiments. Results were analyzed using the Mann-Whitney test. *p < 0.05.

(Figures 3I and 3J). RNA ISH demonstrated that *IFNL1* was expressed primarily within the corneal epithelial cells, similar to what was observed for the *IFNLR1* (Figure 3K). Thus, IFN- λ and IFN λ R1 are expressed in the human corneal epithelium, and signaling through IFN λ R1 restricts replication of HSV-1 in human corneal explants.

It has been widely speculated that SARS-CoV-2 may infect the eye (Xia et al., 2020), although it is unusual for patients with SARS-CoV-2 to develop ocular symptoms. The possibility of corneal infection is supported by the fact that ACE2, a receptor for SARS-CoV-2, is highly expressed in the human corneal epithelium (Collin et al., 2020; Zhou et al., 2020). Therefore, we tested whether a clinical isolate of SARS-CoV-2 could replicate

in human corneal tissue. However, we detected no evidence of SARS-CoV-2 replication using qRT-PCR in seven independent donor samples. The same negative results were obtained by plaque assay (data not shown). Positive controls were used in all experiments, including confirmation that HSV-1 could replicate in portions of the very same donor corneas. Unlike what was observed with HSV-1 and ZIKV, blockade of IFN λ R1 did not make the human corneal tissue more permissive to infection with SARS-CoV-2 (Figure 3L). As the corneal explants also include a small amount of conjunctival tissue, our findings suggest that the human cornea and conjunctiva may not support infection with SARS-CoV-2 and that antiviral immunity against SARS-CoV-2 in the cornea is mediated by a pathway other than IFN λ R1 signaling. Nevertheless, further study of human patient and autopsy samples will be necessary to confirm that the human cornea and conjunctiva are resistant to infection with SARS-CoV-2.

Studies of antiviral immunity at barrier surfaces previously demonstrated that IFN- λ regulates permeability of the blood-brain barrier (Lazear et al., 2015), protects the gut against enteric virus infection (Nice et al., 2015), and promotes antiviral immunity at the placental barrier (Jagger et al., 2017). We discovered that the human corneal barrier also is a site of action for IFN- λ and IFN λ R1 and that IFN λ R1 signaling inhibits replication of HSV-1 in human corneal explants. Additionally, we found that IFN- λ eye drops upregulate antiviral ISGs in WT but not in *Ifnlr1*^{-/-} corneas in mice, revealing a capacity of IFN- λ to exert a topical effect. As the IFN- λ antiviral pathway was not previously described to have activity in the human eye, our discoveries may lead to further exploration of the role of IFN- λ and IFN λ R1 during ocular infection and inflammatory eye disease pathogenesis in humans.

IFN λ R1 signaling likely occurs in corneal epithelial cells. In support of this conclusion, we detected expression of *IFNLR1* and *IFNL1* mRNA within the human corneal epithelium. Corneal epithelial cells also are a primary target for HSV-1 infection of the cornea (Farooq and Shukla, 2012; Oh, 1976), and we found that blockade of IFN λ R1 signaling led to more severe HSV-1 infection of human corneal explants, indicating that IFN- λ signaling in the cornea mediates protection against herpesvirus infection. As our experiments only tested the role of IFN- λ during infection of human corneas, future studies in mice may allow us to examine whether similar anti-viral effects occur in animal models. Although prior studies of the mouse cornea have not examined the effect of IFN λ R1 blockade or genetic deletion, treatment of mice with recombinant IL-28A (IFN- λ) affected immune responses and infection in a model of stromal keratitis (Jaggi et al., 2018), providing further support for the idea that IFN- λ is relevant in the eyes of mice. Future studies in mouse models using Cre-lox alleles for IFN λ R1 and IFN- λ 2/3 will allow us to define the cell type-specific effects of IFN- λ and IFN λ R1 during corneal inflammation and infection.

We previously found that ZIKV infects the corneas of mice lacking IFNAR1 (*Ifnar1*^{-/-} mice) (Miner et al., 2016b). Although we confirmed that ZIKV can be transmitted by corneal transplantation in immunodeficient mice, severe systemic infection occurred in only one of six animals. Furthermore, ZIKV replicated inefficiently in human corneal explants, suggesting that the cornea is unlikely to be a major target of ZIKV infection despite what has been

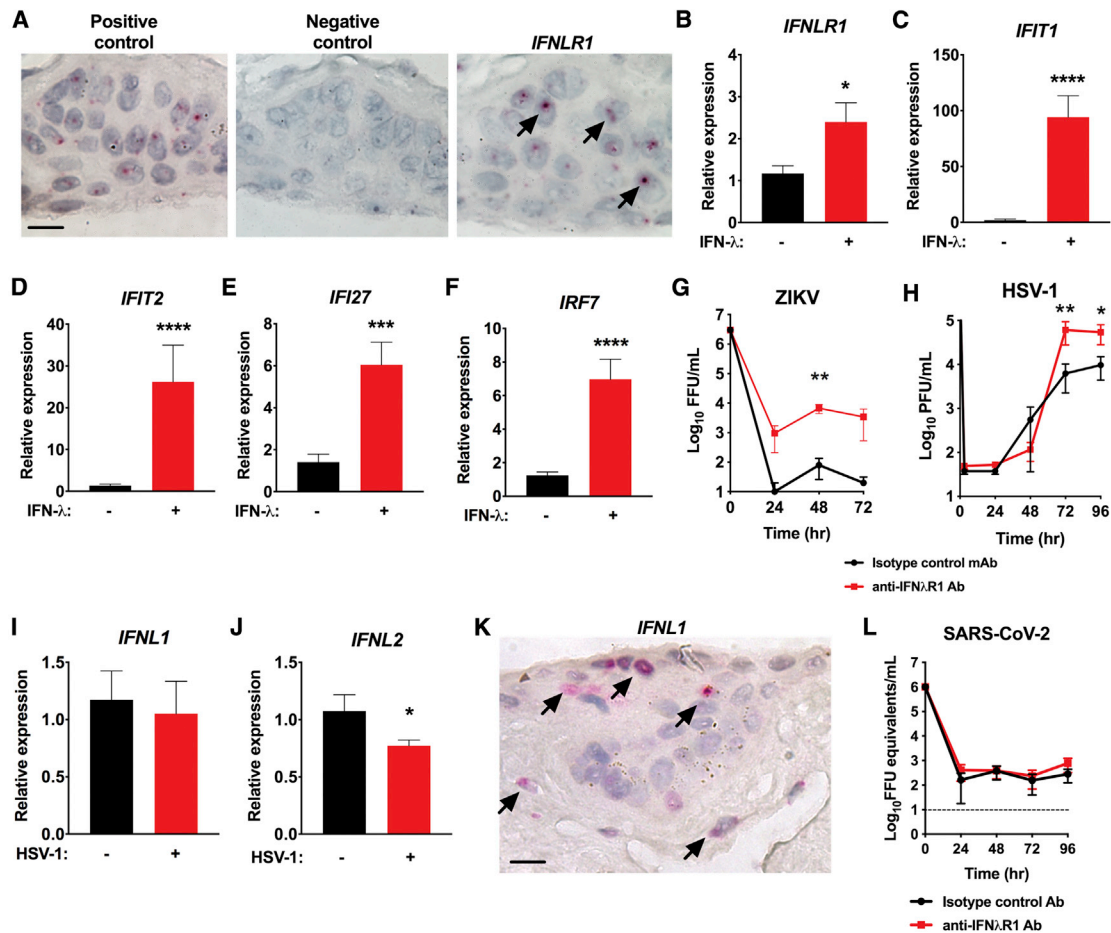


Figure 3. The IFN- λ Receptor (IFN λ R1) Regulates Antiviral ISG Expression and Restricts HSV-1 Growth in Human Corneal Explants

(A) RNA ISH and histological analysis were performed on human donor corneas to determine *IFNL1* mRNA expression in the corneal epithelium. Panels shown are positive control housekeeping gene (left), negative control probe (middle), and *IFNL1* (right). Scale bar, 10 μ m. Data are representative of three independent experiments with unique donor corneas. Black arrows indicate positive staining of cells for *IFNL1*.

(B–F) Human donor corneas were treated with IFN- λ or PBS vehicle control, and ISG expression from human corneal explants was quantitated using qRT-PCR. Data represent the mean \pm SEM of 12 samples, including 4 cornea segments per donor from 3 donor corneas. Results were pooled from two independent experiments.

(G) ZIKV replication in a representative human donor cornea sample treated with anti-IFN λ R1 or isotype control antibody. Infectious units of ZIKV were quantitated by focus forming assay. ZIKV replicated in only 2 of 5 human cornea samples under these conditions. Results were analyzed using two-way ANOVA.

(H) HSV-1 replication in human donor corneas treated with anti-IFN λ R1 or isotype control antibody. Infectious units of HSV-1 were quantitated at the 3 h eclipse phase and every 24 h thereafter by plaque assay. Data represent the mean \pm SEM of $n = 12$ replicates, including 4 technical replicates per group from 3 corneas. Results in (H) were pooled from three independent experiments and were analyzed using two-way ANOVA.

(I and J) *IFNL1* and *IFNL2* mRNA expression levels were quantitated using qRT-PCR 24 h after infection with HSV-1. Data represent the mean \pm SEM of mock-infected ($n = 3$) and infected ($n = 3$) human donor corneas with 4 cornea segments per donor in two independent experiments.

(K) RNA ISH and histological analysis were performed on human donor corneas to determine *IFNL1* mRNA expression in the cornea. Scale bar, 10 μ m. Data are representative of 10 samples from seven independent donors. Samples from two donors repeatedly did not stain for positive control (or target) and were excluded. Black arrows indicate positive staining of cells for *IFNL1*.

(L) SARS-CoV-2 replication in human donor corneas samples treated with anti-IFN λ R1 or isotype control antibody. SARS-CoV-2 mRNA expression was measured using qRT-PCR. Data represent the mean \pm SEM of $n = 7$ –11 replicates, with two to four technical replicates per group from 3 donor corneas. Results in (L) were pooled from two independent experiments and were analyzed by two-way ANOVA.

Results in (B)–(F), (I), and (J) were pooled from two independent experiments and were analyzed using the Mann-Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. See also Figure S1.

observed in animal models. Nevertheless, ZIKV RNA has previously been detected in the vitreous humor of a human donor sample (Heck et al., 2017) and in human conjunctival swabs (Sun et al., 2017), suggesting that ZIKV can occasionally be harbored in or

associated with human corneas. As even one infection occurring as a consequence of corneal transplantation could be harmful, there is a need to continue screening donated corneas for arboviruses, especially in the context of virus outbreaks.

We discovered that HSV-1 has the capacity to antagonize production of IFN- λ 2 in the human cornea, implying that immune evasion of IFN- λ may contribute to HSV-1 disease pathogenesis. Prior cell culture studies demonstrated that IFN- λ inhibits HSV-1 replication in astrocytes and neurons by stimulating the expression of endogenous type I IFN and ISGs (Li et al., 2011). Our study confirms that IFN λ R1 signaling restricts HSV-1 in human samples and also reveals a role for this pathway in the human eye.

In addition to expression in corneal epithelial cells, IFN λ R1 is expressed on neutrophils, which are known to infiltrate and damage the cornea during HSV-1 infection (Thomas et al., 1997). Remarkably, in addition to controlling infection, IFN- λ can ameliorate inflammatory responses mediated by neutrophils (Blazek et al., 2015). Thus, our work may inform further studies of disease pathogenesis in animals, as well as therapies targeting IFN λ R1. This may eventually lead to prophylactic or therapeutic administration of IFN- λ to control infection or to dampen destructive immune responses in the cornea.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.celrep.2020.108339>.

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AUTHOR CONTRIBUTIONS

D.J.P., P.C., A.S., A.M.M., Z.D., W.Q., and E.S.K. performed experiments. J.J.M., D.J.P., A.S., A.M.M., Z.D., and E.S.K. analyzed data. C.M.G. and E.R.W. analyzed data and wrote portions of the initial manuscript and edited subsequent versions of the manuscript. J.J.M., D.J.P., and E.R.W. wrote the final complete version of the manuscript. J.J.M., J.A.P., and R.S.A. conceived the project, analyzed data, and edited the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-human interferon lambda receptor 1 neutralizing mAb	PBL assay science	Cat# 21885-1 Unable to locate RRID
Mouse IgG1 kappa isotype control mAb	Sigma-Aldrich	Cat# M7894, RRID:AB_1163632
Bacterial and Virus Strains		
Herpes simplex virus-1 strain 17+	Bertke et al., 2013	N/A
Zika virus, strain Paraiba 2015 (Brazil, 2015)	Magnani et al., 2018	N/A
SARS-CoV-2 (strain 2019 n-CoV/USA_WA1/2020)	CDC/BEI Resources	NR52281
Biological Samples		
Human corneas	Mid-America Transplant Institute	N/A
Chemicals, Peptides, and Recombinant Proteins		
Recombinant Human IL-29/IFN Lambda 1, HCE	PBL assay science	Cat# 11725-1
Recombinant Mouse IL-28B/IFN Lambda 3	PBL assay science	Cat# 12820-1
Critical Commercial Assays		
RNeasy Mini Kit	QIAGEN	Cat# 74104
TaqMan RNA-to-Ct 1-Step Kit	ThermoFisher	Cat# 4392653
Experimental Models: Cell Lines		
Vero CCL81 cells	ATCC	CCL81; RRID: CVCL_0059
Vero E6 cells	ATCC	CRL-1586; RRID: CVCL_0574
Experimental Models: Organisms/Strains		
Mouse: <i>Ifnar1</i> ^{-/-}	Jackson Laboratory	Cat# JAX:032048, RRID: IMSR_JAX:032048
Mouse: AG129	Marshall BioResources	AG129
Mouse: <i>Ifnlr1</i> ^{-/-}	Baldridge et al., 2017	N/A
Oligonucleotides		
SARS-CoV-2 N F: 50 -ATGCTGCAATCGTGCTACAA-3	Hassan et al., 2020	N/A
SARS-CoV-2 N R: 50 -GACTGCCGCCTCTGCTC-30	Hassan et al., 2020	N/A
SARS-CoV-2 N Probe: 50 -/56-FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/-30	Hassan et al., 2020	N/A
RNAscope® Probe- V-ZIKV	Advanced Cell Diagnostics	Cat# 467771
RNAscope® Probe- Hs-IFNLR1	Advanced Cell Diagnostics	Cat# 494771
RNAscope® Probe- Hs-IFNL1	Advanced Cell Diagnostics	Cat# 412341
<i>IFNL1</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs.PT.56a.21113836.g
<i>IFNL2</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs.PT.56a.38564463
<i>IFNLR1</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs00417120_m1
<i>IFIT1</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs.PT.56a.20769090.g
<i>IFIT2</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs.PT.58.1099131
<i>IRF7</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs.PT.58.24613215.g
<i>IFI27</i> PrimeTime qPCR Assay	ThermoFisher	Hs01086373_g1
<i>GAPDH</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Hs.PT.39a.22214936
<i>Ifit1</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Mm.PT.58.32674307
<i>Ifit2</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Mm.PT.58.45910930.g
<i>Ifitm3</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Mm.PT.56a.29591636
<i>Irf7</i> PrimeTime qPCR Assay	ThermoFisher	Mm00516793

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>Isg15</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Mm.PT.58.41476392
<i>Iff127</i> PrimeTime qPCR Assay	ThermoFisher	Mm00835449_g1
<i>Gapdh</i> PrimeTime qPCR Assay	Integrated DNA Technologies	Mm.PT.39a.1
Software and Algorithms		
GraphPad Prism 8	GraphPad	https://www.graphpad.com

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jonathan J. Miner (miner@wustl.edu).

Materials Availability

This study did not generate new unique reagents. Commercially available reagents are indicated in the Key Resources Table.

Data Availability

This study did not generate/analyze datasets/code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines

Vero CCL81 cells were used to passage and titrate ZIKV and HSV-1. Vero E6 cells were used to passage and titrate SARS-CoV-2. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin/streptomycin, 10mM HEPES buffer, 1mM sodium pyruvate, and 1 x non-essential amino acids. Cell lines were kept at 37°C in a humidified atmosphere at 5% CO₂.

Viruses

ZIKV (Brazil strain) was a passage 3 stock derived from a clinical isolate from Paraiba, Brazil ([Magnani et al., 2018](#)). Viral titers were measured by focus forming assay as previously described ([Miner et al., 2016a](#)). ZIKV was passaged in Vero CCL81 cells. HSV-1 strain 17+ also was passaged in Vero CCL81 cells and provided by T. Margolis (Washington University, Saint Louis, MO) ([Bertke et al., 2013](#)). SARS-CoV-2 (2019 n-CoV/USA_WA1/2020) was obtained from the Centers for Disease Control and Prevention (gift of Natalie Thornburg) and was passaged in Vero CCL81 cells. SARS-CoV-2 titers were measured by qRT-PCR and by infectious plaque assay in Vero E6 (CRL-1586) cells ([Hassan et al., 2020](#)).

Mouse experiments

All protocols for animal studies were approved by the Institutional Animal Care and Use Committees at the Washington University School of Medicine (assurance no. A-3381-01). Mice were housed in specific pathogen-free mouse facilities at the Washington University School of Medicine. AG129 and *Ifnar1*^{-/-} mice were purchased from the Jackson laboratory. *Ifnl1*^{-/-} mice were previously described ([Baldrige et al., 2017](#)) and provided by M.T. Baldrige (Washington University, Saint Louis, MO). Power analysis was conducted for Institutional Animal Care and Use Committee-approved *in vivo* studies in order to determine the number of animals needed per experimental group. At least two independent experiments were conducted to replicate findings. No outliers were excluded from analyses. Approximately equal numbers of both sexes were studied. The age and number of animals used for each experiment is listed in the figure legends.

Ifnar1^{-/-} mice were inoculated with 10³ FFU of a pathogenic clinical ZIKV isolate from Brazil via subcutaneous footpad inoculation. Corneas were harvested 7 days post-inoculation and homogenized. Eight-week-old AG129 mice were then inoculated intraperitoneally with corneal homogenates in 100 μL of PBS or mock-infected cornea control samples. Mice were monitored for 30 days to assess survival. For corneal transplantation studies, AG129 mice were inoculated subcutaneously with 10³ FFU of ZIKV. Donor mice were euthanized 7 days post-infection and corneas were microdissected and washed repeatedly in PBS, and then transplanted to AG129 recipients as previously described ([He et al., 1991](#)). Recipient mice were euthanized 7 days post-transplantation, followed by dissection of tissues, homogenization, and quantitation of viral RNA by qRT-PCR using virus-specific primers and probes as previously described ([Miner et al., 2016a](#)).

METHOD DETAILS

Human cornea infections and virus stability measurements

De-identified human donor corneas were obtained from Mid-America Transplant Institute and consisted of cornea samples found to be unsuitable for transplant. Experiments were performed on a total of 25 donor corneas (ages of donors were 20-, 21-, 23-, 28-, 32-, 35-, 40-, 40-, 40-, 40-, 40-, 51-, 53-, 55-, 55-, 55-, 56-, 56-, 64-, 66-, 66-, 67-, 67-, 69-, and 74-years-old) from both genders. This work was IRB-exempt since it involved de-identified human tissue from deceased organ donors.

For ZIKV replication studies, human corneal explants were inoculated with 3×10^6 FFU/ml of ZIKV. Corneas were washed 6 times in 50 mL of PBS, and growth curve analysis was performed by focus forming assay as previously described (Miner et al., 2016a). For growth curve experiments examining HSV-1, ZIKV, and SARS-CoV-2 replication in the presence and absence of IFN λ R1 blockade, anti-IFN λ R1 mAb (PBL assay science, catalog # 21885-1) and isotype control antibody (Cat# M7894, RRID:AB_1163632), were used at a concentration of 10 μ g/ml with fresh antibody introduced every 24 hours. In 2 of 5 samples, the initial HSV-1 infection failed, and so these samples were excluded. For SARS-CoV-2 infection studies, the inoculation dose was 1×10^6 PFU/ml, followed by washing steps as described for HSV-1 and ZIKV. Seven human donor samples were evaluated for SARS-CoV-2 infection, three of which were evaluated in the presence of anti-IFN λ R1 mAb or control mAb. Treatment of human corneas with recombinant human IL-29/IFNL1 (PBL assay science, catalog #11725-1) was performed at a concentration of 4.35×10^4 U/ml for 6 hours. Cornea storage medium (OptisolTM) was obtained from the Washington University Department of Ophthalmology. Persistence of infectious ZIKV in cornea storage medium was assessed by introducing 6×10^5 FFU of ZIKV into cornea storage medium at 4°C. Levels of infectious ZIKV were quantitated by focus forming assay every two days for 28 days.

RNA *in situ* hybridization (ISH) and ISG expression

Human corneas were sectioned and RNA ISH was performed with RNAscope[®] 2.5 (Advanced Cell Diagnostics) according to the manufacturer's instructions, using virus-specific probes for ZIKV as previously described (Miner et al., 2016b). To visualize *IFNLR1* and *IFNL1* mRNA expression in uninfected human corneas, samples were sectioned and RNA ISH was performed with specific probes for the *IFNLR1* gene using ACD Bio probe number 494771 RNAscope[®] 2.5 (Advanced Cell Diagnostics) Probe-Hs-IFNLR1. RNA Scope 2.5 HD red was used per manufacturer's instructions.

Gene expression analysis and IFN- λ treatment of mice

Total RNA was extracted from microdissected corneal homogenates using the RNeasy kit (QIAGEN) according to the manufacturer's instructions. To test whether ISG upregulation was dependent on IFN λ R1, we administered two doses of 1 μ g of mouse IL28B/IFN- λ 3 in PBS (PBL assay science, catalog #12820-1) or PBS control to WT and *Ifnlr1*^{-/-} mice. Doses were 3 hours apart. Three hours after the 2nd treatment, mice were euthanized and corneas were harvested. qRT-PCR was performed using TaqMan RNA-to-Ct 1-Step kit (Applied Biosystems) according to the manufacturer's instructions. Results were calculated using the $\Delta\Delta C_t$ method, normalizing to GAPDH. Primers were obtained from Integrated Device Technology (IDT).

Human cornea inhibitor experiments

Human corneas were cut into 8 pieces per cornea and placed individually into 24-well plates with 0.3mL media containing inhibitor or vehicle control. Inhibitors used were 10 μ M MRT67307 (Selleckchem S7948) and 50 μ M azidothymidine (R&D Systems 4150/50). For each donor, 4 pieces from one cornea were incubated with the inhibitor and the remaining 4 pieces with vehicle for 6 hours at 37°C. Cornea pieces were stored at -80°C. After thawing, pieces were transferred to gentleMACS C tubes containing 1mL DMEM + 2% FCS + collagenase (Sigma, C0130) + dispase I (Sigma D4818) and incubated with shaking at 37°C for 2 hours. Tissues were dissociated using a gentleMACS Dissociator (Miltenyi). Dissociated tissue was then filtered over 70 μ m mesh filters, centrifuged at 300 x g for 10 min at 4°C, and pellets were resuspended in 250 μ L RLT buffer (QIAGEN). RNA was isolated using the RNeasy mini kit (QIAGEN) according to the manufacturer's protocol. qRT-PCR was performed using a TaqMan RNA-to-C_T 1-step kit (Applied Biosystems). Results were calculated using the threshold cycle ($\Delta\Delta C_T$) method, normalizing to GAPDH. Primers were obtained from Integrated Device Technology, Inc. (IDT).

QUANTIFICATION AND STATISTICAL ANALYSIS

All data were analyzed using GraphPad Prism software by Mann-Whitney test or two-way ANOVA, as specified in the figure legends.

Cell Reports, Volume 33

Supplemental Information

HSV-1 and Zika Virus but Not SARS-CoV-2

Replicate in the Human Cornea and Are

Restricted by Corneal Type III Interferon

Jonathan J. Miner, Derek J. Platt, Cyrus M. Ghaznavi, Pallavi Chandra, Andrea Santeford, Amber M. Menos, Zhenyu Dong, Erin R. Wang, Wei Qian, Elysse S. Karozichian, Jennifer A. Philips, and Rajendra S. Apte

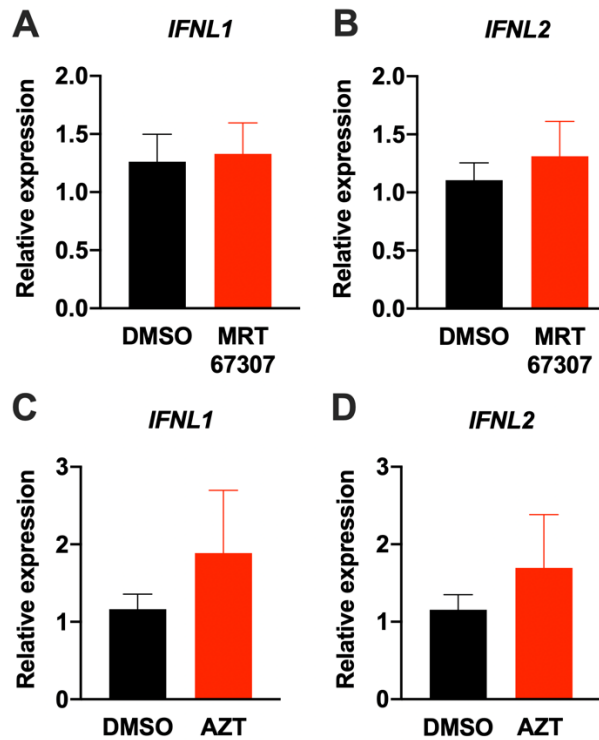


Figure S1. Treatment with inhibitors of TBK1 or reverse transcriptase does not impact expression of *IFNL1* or *IFNL2* in the human cornea. Related to Figure 3. (A-D) Human donor corneas were treated with TBK1 inhibitor (MRT 67307), an anti-retroviral drug (AZT), or DMSO vehicle control for 6 hours, and then *IFNL1* and *IFNL2* mRNA expression was quantitated by qRT-PCR. Data represent the mean \pm SEM of human donor corneas ($n = 3$) with 4 cornea segments per condition pooled from 2 independent experiments. Results were analyzed by Mann-Whitney test.