

RESEARCH ARTICLE

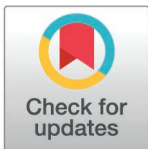
Comparative susceptibility of Old World and New World bat cell lines to Zika virus: Insights into viral replication and inflammatory responses

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Data availability statement: The code to reproduce the analyses and visualization is available through GitHub (<https://github.com/viralemergence/zika-rnaseq-analysis>). Illumina reads are available from the sequencing read archive (SRA, accession: PRJNA1241890)

Abstract

Background

The emergence of flaviviruses and other arboviruses in novel geographic locations, arthropod vectors, and vertebrate amplification hosts complicates control and eradication efforts. Many flaviviruses continue to impact global health, including Zika virus (ZIKV) which has expanded to a global health threat, yet many questions remain surrounding its ecology and inter-epidemic maintenance. While bats are known to harbor several medically important viruses without clinical disease, their role in the sylvatic transmission cycle of arboviruses remains unresolved. Reports describing the susceptibility of different bat species to infection with ZIKV are inconsistent, and the immunological mechanisms underpinning this variability have not been well-characterized.

Methodology/Principal Findings

We compared the permissiveness and immune responses of cell lines derived from two frugivorous bat species: the Egyptian fruit bat (*Rousettus aegyptiacus*) and Jamaican fruit bat (*Artibeus jamaicensis*). Using multi-step growth curves, we compared infection dynamics of two genetically distinct ZIKV strains, African lineage (ZIKV/MR766) and Asian-American lineage (ZIKV/PRVABC59) and compared transcriptomic responses to both lineages in susceptible bat-derived cell lines.

Conclusions/Significance

Our results highlight species-specific differences in ZIKV susceptibility across bat-derived cell lines, with both ZIKV strains replicating to high titers on *R. aegyptiacus*

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cells but not on *A. jamaicensis* cells. We detected pro-inflammatory transcriptomic signatures in *R. aegyptiacus* cells infected with both ZIKV lineages. Notably, both the Asian-American and African ZIKV lineages demonstrated some capacity for immune evasion and productive replication in the *R. aegyptiacus* cell line.

Author summary

This study investigates how different lineages of Zika virus (ZIKV) interact with bat cells, specifically examining the cellular inflammatory and antiviral responses. We compare host-virus interactions using cell lines derived from two frugivorous bat species (*Rousettus aegyptiacus* and *Artibeus jamaicensis*). Our results highlight differences in cellular permissiveness and spur additional questions surrounding how infection outcomes are shaped by host taxonomy, viral lineage, and host-virus interactions at the cellular level. Surprisingly, cells derived from *R. aegyptiacus*, the reservoir host of Marburg virus, revealed pro-inflammatory transcriptomic signatures that reflect what is seen during acute infection of humans and non-human primates. African lineage ZIKV (MR766) caused a more robust immune response than Asian-American lineage ZIKV (PRVABC59). In contrast, our *A. jamaicensis*-derived cells appeared resistant to infection with both ZIKV lineages. Our data highlight the importance of comparing host-virus interactions across possible host species and suggest that events occurring after viral entry may shape infection outcomes.

Introduction

The vector borne Zika virus (ZIKV), in the family Flaviviridae, is an emerging global health concern due to its broad geographic distribution and association with congenital microcephaly and Guillain-Barre syndrome [1,2]. Since the virus was first isolated in Uganda in 1947, ZIKV had primarily been associated with sporadic human cases in Africa [3,4]. However, in the early 2000s, the Asian-American lineage began to emerge in larger outbreaks in the Pacific Islands before emerging as an epidemic in the Americas 2015 [5,6]. The African lineage of ZIKV results in higher pathogenesis than the Asian-American lineage, including elevated levels of inflammatory cytokines (e.g., interleukin 6) and tumor necrosis factor [7–9]. The African ZIKV lineage is more frequently associated with the Guillain-Barre syndrome, a neurological manifestation of ZIKV, in infected adults [9,10]. While considered less virulent, the Asian-American lineage is associated with higher incidence of congenital microcephaly [10].

Repeated outbreaks in the Americas following the emergence of ZIKV highlight the importance of identifying mechanisms of continued circulation during inter-epidemic periods [11]. Mathematical modeling of ZIKV outbreaks indicates a sylvatic transmission cycle involving wildlife hosts in addition to human hosts [12,13]. While both Old World and New World nonhuman primates (NHPs) are susceptible to ZIKV

[14,15], outbreaks of Zika virus disease in South America have occurred in areas without molecular detection of ZIKV in NHPs(12). Bats have been posited as an alternative sylvatic reservoir for ZIKV as several species roost in or near human dwellings and there is evidence of bat exposures to arboviruses [16–19] including ZIKV [4]. However, data on the circulation, pathology, and susceptibility of bats to ZIKV are unclear, particularly in the Americas where ZIKV has only recently been introduced.

Early experimental work examining susceptibility of Ugandan bat species to ZIKV suggests they develop low levels of viremia in the absence of clinical disease. Inoculation of an Angolan fruit bat (*Myonycteris angolensis*) indicated a low level of viremia up to 6 days post-infection (DPI) [17]. In another study, low levels of viremia and seroconversion were detected in straw-coloured fruit bats (*Eidolon helvum*) and Egyptian rousette bats (*Rousettus aegyptiacus*) intraperitoneally inoculated with ZIKV [18]. In a subsequent study, ZIKV-reactive antibodies were detected in both bat taxa by a hemagglutination inhibition assay [18]. Detection of ZIKV subgenomic flavivirus RNA (sfRNA) in splenic samples of Ugandan fruit bats including straw-coloured fruit bats, Ethiopian epauletted (*Epomophorus labiatus*) fruit bats and *R. aegyptiacus* suggests previous ZIKV infection, though questions remain surrounding the relationship of sfRNA to the timing of viral infection and/or clearance [4]. Serum from these bats did not contain neutralizing antibodies against ZIKV or other flaviviruses [20]. Experimental inoculation of insectivorous bats including Angolan free-tailed bats (*Mops condylurus*) with ZIKV did not result in viremia, but serosurveillance data indicates a high seroprevalence for ZIKV exposure in both the Angolan and the little free-tailed bat (*Mops [Chaerephon] pumilus*) [17,18,21]. Thus, data on susceptibility of Old World bat species to ZIKV is limited and largely inconclusive.

Surveillance studies examining neotropical bats and other small mammals for evidence of ZIKV infection have increased since the 2015 introduction of the Asian-American lineage of ZIKV. Viral RNA has been detected in common vampire bats (*Desmodus rotundus*) and Jamaican fruit bats (*Artibeus jamaicensis*, JFBs) in Mexico [22,23], though a more recent study screening 162 bats across 23 species found no evidence of ZIKV infection by quantitative reverse-transcription polymerase chain reaction [24]. A short sequence fragment of the ZIKV NS5 gene, associated with the flat-faced fruit bat (*Artibeus planirostris*) from Grenada, West Indies, was deposited in GenBank (Accession #MH255606) in 2018, though this sequence has not been linked to a peer-reviewed publication. Large surveys across Central and South America, including Brazil, French Guiana, Peru, and Costa Rica, have not detected ZIKV nucleic acids in bats [24–26].

Studies with experimental challenge *in vivo* also suggest limited susceptibility of neotropical bats to ZIKV. Inoculation of bats in the genus *Artibeus* resulted in minimal pathology with variable detection of viral RNA in brain, urine, and saliva but viremia was not observed [27]. A follow-up study reported detection of ZIKV subgenomic flavivirus RNA (sfRNA) in blood and multiple organs of JFBs up to six weeks post-inoculation with three different ZIKV strains [4]. Inoculation of the great fruit-eating bat (*Artibeus lituratus*) with an Asian-American lineage ZIKV isolate did not result in detectable viremia or the production of neutralizing antibodies, though ZIKV RNA was detected in two of nine inoculated bats [25]. While these findings suggest that New World bats may have limited susceptibility to ZIKV, evidence that they develop viremia sufficient to contribute to viral transmission is lacking.

To characterize the ecological and immunological dynamics of ZIKV in bat reservoirs, we conducted comparative transcriptomic analyses *in vitro*. We explicitly tested the hypothesis that New World bat cells exhibit lower permissiveness to ZIKV infection and/or heightened innate immune activation relative to Old World bat cells, reflecting differences in evolutionary history with ZIKV, and that these cellular phenotypes correlate with ecological observations of limited ZIKV circulation in New World bat populations. We hypothesized that Old World bat species, having co-existed with ZIKV over extended evolutionary timescales, may exhibit signatures of immune tolerance, paralleling patterns observed with *Rousettus aegyptiacus* and Marburg virus. In contrast, New World bats such as *Artibeus jamaicensis* have only encountered ZIKV since the recent introduction of the Asian-American lineage to the Americas roughly a decade ago. Both *R. aegyptiacus* and *A. jamaicensis* are common and widespread within their respective geographic ranges, making them ecologically relevant representatives of bat-virus interfaces in the Old and New Worlds. Given their frequent presence at

human-animal interfaces, bats are plausible amplifying hosts, yet a central question remains: Are bats susceptible to ZIKV infection? Viral susceptibility at the host-cell level requires the presence of appropriate entry factors, intracellular machinery to support viral replication, and the ability of the virus to evade or suppress host innate immune defenses. Here, we compare the *in vitro* susceptibility and transcriptional responses of bat cell lines derived from *R. aegyptiacus* and *A. jamaicensis* following infection with African and Asian-American ZIKV lineages, providing insight into how evolutionary history and ecological context shape host-virus interactions.

Materials and methods

Ethics statement

All experimental work with Zika virus was conducted under appropriate biosafety containment levels (e.g., BSL2+) at Washington State University as approved by the Institutional Biosafety Committee.

Cell culture and maintenance

We cultured *Artibeus jamaicensis* immortalized kidney cells (Aji cells), derived from renal tissue and immortalized using a lentiviral vector expressing SV40 T-antigen as previously described [28], and immortalized *R. aegyptiacus* fetal cell line (R06E, BEI #NR-49168) [29]. The R06E cell line was generated from primary cells isolated from the body of an Egyptian fruit bat (*R. aegyptiacus*) fetus and immortalized by transfection with an adenovirus type 5 E1A/E1B expression plasmid, according to BEI Resources documentation. Tissue of origin and sex were not specified by the supplier. Species identity of both cell lines was independently confirmed in our laboratory by cytochrome b sequencing, and all cultures tested negative for mycoplasma contamination prior to experimentation. Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 14% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% L-glutamine, 1% non-essential amino acids (NEAA), and 1% sodium pyruvate. We maintained cells at 37°C with 5% CO₂. Experiments were performed using low-passage cells to minimize cell culture-associated artefacts.

Comparative viral growth curves

We conducted viral growth curves using R06E cells and Aji cells by inoculating each cell line at a multiplicity of infection (MOI) of 0.01 with Zika virus (ZIKV) isolates ZIKV/MR766 or ZIKV/PRVABC59 in quadruplicate for each time point and incubated for one hour at 37 °C and washed with PBS. We collected supernatant at 0, 24, 48, and 72 hours post-inoculation (HPI). We then conducted back titrations to quantify the 50% tissue culture infectious dose (TCID₅₀/mL) of the inoculum.

We analyzed viral growth curves using repeated measures ANOVA to compare TCID₅₀/mL values between cell lines (Aji vs R06E), virus strains (MR766 vs PRVABC59), and across time points (0, 24, 48, and 72 hours post-infection). TCID₅₀ values were log₁₀-transformed prior to analysis to achieve normality. Prior to analysis, we tested for homogeneity of variance using Levene's test and assessed normality of residuals using Shapiro-Wilk tests and Q-Q plots. We conducted post-hoc pairwise comparisons between virus strains within each cell line and time point using estimated marginal means with Tukey adjustment for multiple comparisons. We performed all statistical analyses in R version 2025.09.2 + 418 using the car [30] and emmeans [31] packages with significance set to $\alpha = 0.05$.

In vitro inoculation for transcriptomics

We used two ZIKV isolates in our experiment: MR766 (African lineage, BEI #NR-50065) and PRVABC59 (Asian-American lineage, BEI #NR-50240). For each virus-cell line combination, we generated three biological replicates and one negative control (PBS-inoculated) in 48-well plates. To synchronize infection across the cell population, we inoculated all samples at an MOI of 1 and collected samples at four timepoints: 6, 12, 24, and 48 HPI. At each timepoint, we collected infectious

virus from the supernatant and scraped TRIzol-inactivated cells from the well surface. We incubated plates for timepoints 6, 12, 24, and 48 HPI at 37 °C with 5% CO₂. We used infectious samples for back titrations and processed inactivated cells for RNA sequencing (RNA-seq).

We extracted RNA from TRIzol-inactivated cells using either TRIzol (Invitrogen) for manual phase separation or the Direct-zol 96 RNA extraction kit (Zymo) following the manufacturer's instructions. We prepared total RNA-seq libraries using the Zymo-Seq RiboFree Total RNA Library Kit (Zymo Research), performed fragment analysis for quality control, and sequenced libraries on an Illumina NextSeq 2000 as paired-end reads (2 × 100 bp). Samples were meant to have 3 biological replicates, but due to low quality and other technical issues, many replicates were removed. Most samples maintained 2–3 replicates, but 3 samples ended with only 1 replicate: 2 of the Aji samples and 1 of the R06E samples. [S2 Table](#) has the complete replicate counts, with column 1 listing "{cell line}-{virus treatment}-{timepoint}" in this format, and column 2 listing the counts.

RNA-seq read processing and mapping

RNA sequencing reads were first processed by running them through the fastp tool [32], with adapter removal (see code for command). The trimmed reads underwent QC by subsequently running them through FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and the output graphs were manually inspected. All samples appeared acceptable at this step.

Reads were then mapped to their respective genomes with corresponding annotations from NCBI. For *Rousettus* the GCF_014176215.1_mRouAeg1.p_genomic genome [33] and annotations were used, and for *Artibeus* the GCF_021234435.1_CSHL_Jam_final_genomic genome [34] and annotations were used. The mapping tool STAR [35] was used to build genome indices from the respective genome and annotation files. Consequently, when STAR mapping was performed it was able to provide gene counts concurrently with read mapping, removing the need for an additional gene counting step.

Gene count processing, batch correction, and normalization

Several steps were carried out for initial gene count processing. First, 6 samples had technical replicates, so the resulting gene counts were combined within technical replicates. Next, manual inspection revealed 2 samples exhibited huge proportions of missing or skewed genes, so these were removed from subsequent analyses. Lastly, the gene counts were collated into a single table. These steps were facilitated by the `gene_count_and_metadata_processing.py` script.

The batch effect of RNA-seq library preparation was then corrected using pyComBat [36]. Since pyComBat builds a comprehensive model to quantify and remove batch effect, we chose to process gene counts by cell line to reduce runtime and computational load. However, within each cell line we did demarcate virus treatment and timepoints as covariates in pyComBat so their effects would not be lost during correction.

Finally, gene counts were normalized using PyDESeq2 [37]. The resulting normalized gene counts are returned in the code as a python object, by which several analyses may be performed, including differentially expressed gene (DEG) identification and principal component analysis. However, for DEG identification and p-value calculations, the current PyDESeq2 module lacks granularity for multifactor models. For instance, it can calculate DEGs with p-values between virus treatment contrasts (e.g., no treatment vs MR766 treatment) or timepoint contrasts (e.g., 6 HPI vs 12 HPI), but not both (e.g., 6 HPI no treatment vs 6 HPI MR766 treatment). Consequently, depending on the analysis we performed, the data was parsed to the necessary level of granularity before normalization. This is specified below, per analysis.

Principal component analysis

For the principal component analysis (PCA), gene counts were parsed by cell line and virus treatment contrast, then normalized with PyDESeq2. The resulting counts underwent PCA using the scanpy module [38].

Spearman correlations

For Spearman correlations, the gene counts were parsed only by cell line, then normalized with PyDESeq2. The mean gene count values were calculated for replicates, and then any genes with a mean count of 0 for all samples were dropped to prevent artificially inflating the correlation due to genes that may simply not be expressed in the cell lines. Finally, the curated gene count means were used for pairwise Spearman correlations. The coefficients were rounded to 4 significant figures and plotted.

Differentially expressed gene identification

To appropriately calculate DEG $\log_2$ fold changes and p-values in our multifactor design, the gene counts were parsed by cell line, virus treatment contrasts, and then timepoint, and the subsets normalized with PyDESeq2 (e.g., 'R06E 6 HPI no treatment' vs 'R06E 6 HPI MR766 treatment'). Then, using the PyDESeq2 `DeseqStats()` function, $\log_2$ fold changes and Wald test p-values were calculated. These p-values were adjusted for multiple hypotheses using the Benjamini-Hochberg procedure implemented in PyDESeq2. Throughout this manuscript, references to 'p-values' from differential expression analysis refer to these adjusted values. Depending on the analysis, the DEGs were filtered by $\log_2$ fold change and p-value, as stated below.

Gene set enrichment analysis

The $\log_2$ fold changes and p-values previously calculated were used for gene set enrichment analysis (GSEA) without filtering for non-DEGs (i.e., all genes were used). All genes were ranked, per gene, using the equation:

$$\text{rank} = -\log_{10}(\text{p-value}) * \log_2 \text{fold change}$$

Thus, both the fold change and the p-value informed how enriched a gene was amongst all genes. However, because a gene set is not necessarily a pathway, and at times may exhibit both up and down expression, we chose to perform GSEA separately for up and down regulated genes. When analyzing downregulated genes, we multiplied the $\log_2$ fold change by negative 1 to make the resulting enrichment scores positive. Lastly, the ranked genes were scaled by dividing all rank values by the max value, thus making the new scaled max value always 1. This had no effect on the order of genes or the enrichment statistics; it was primarily done to make direct comparison of rank across virus contrasts more intuitive (e.g., the scaled ranks can be seen in Fig 5A, running from 1 to 0).

The ranked genes were then used as input for the GSEAPy module [39], which calculated normalized enrichment scores (NESs) and false discovery rates (FDRs) per gene set. FDRs were calculated using the Benjamini-Hochberg correction method. Gene sets with $\text{FDR} < 0.05$ were considered significantly enriched. We used the KEGG_2021_Human gene sets [40], as there were no defined KEGG bat gene sets for the module (though there may be elsewhere) and bats are not a model organism, whereas the human genome is well characterized, and the human gene sets well-curated. Enriched gene sets were calculated for all contrasts and timepoints, but we chose to highlight only the top 10 gene sets with the lowest FDRs for 6 HPI (per virus contrast) and subsequently depict the NESs for these gene sets across all time points (Fig 2).

Gene ontology enrichment analysis

The DEGs were filtered for gene ontology enrichment analysis (GOEA) by selecting genes with an absolute $\log_2$ fold change greater than 1.5 and p-value of less than 0.05. GO term enrichment p-values reported by goatools represent the statistical significance of each term's enrichment within the input DEG set. The remaining genes were used as input for the goatools python module [41] to perform GOEA. The GO term tree was built using the main go.obo file from the geneontology.org website (see code for ftp path) and DEG IDs were mapped to GO terms using the gene2go.txt file from the

NCBI (see code for ftp path) and taxon ID 9407. Lastly, a file containing all *Rousettus* genes was acquired from the NCBI using the search query:

"9407"[Taxonomy ID] AND alive[property] AND genotype protein coding[Properties]

This file was used by goatoools as the baseline "background genes" set for determining which GO terms were biologically enriched and not enriched by chance.

The enriched GO term results from the primary GOEA were then mapped to the GO slim terms in the goslim_pir.obo file from the geneontology.org website. This allowed us to group enriched GO terms and reduce redundancy; however, the GO slim terms (representing subsets of the full ontology) tend to be broad and high-level. For our analyses and interpretation, we took the GO term with the most significant p-value in each GO slim group and chose that to represent the entire group (see S2 Fig). In most cases, the most enriched term was not the GO slim term, but in the rare cases it was, we chose the second most enriched term to increase interpretability. These are the GO terms used in the paper and figures.

DEG expression pattern identification

As per the GOEA, DEGs were filtered so remaining genes had an absolute $\log_2$ fold change greater than 1.5 and p-value of less than 0.05. The counts for these genes were then clustered by the R package DEGreport [42]; more specifically, the degPatterns() function. Default parameters were used, including a minimum count of 15 genes per group for a group to be reported and a clustering coefficient cutoff of 0.7. Consequently, only 3 viable groups were generated, as shown in Fig 4.

Protein-protein interaction network analysis

The 6 HPI upregulated genes (see Fig 5B) in the KEGG "Viral Protein Interaction With Cytokine and Cytokine Receptor" gene set were submitted to the STRING database version 12.0 [43] via the STRING API with default parameters. The API request returned a list of gene interactions with scores representing the likelihood of interaction based on different factors such as literature sources describing interaction. The interactions were used to generate a protein-protein interaction (PPI) network, where the edges were interactions between genes and the weights of the edges were the interaction scores pulled from the database. The genes were examined for interaction counts (i.e., number of edges) and the genes with the highest counts were identified as hub genes; the most interconnected genes of the network which play potentially central roles. The interaction count was also depicted graphically (Fig 5C). The network was generated using the NetworkX module [44] and the Fruchterman-Reingold force-directed algorithm (encapsulated in the spring_layout() function).

Data analysis and visualization

The bioinformatic analyses were performed or orchestrated by in-house code, which can be found in the GitHub repo below. Additionally, they were run in a Singularity container made from a Docker image with the build file in the same repo. The graphs and other data visualizations presented in the paper were either generated using the modules for each respective analysis (e.g. GSEAPy), or the same previously mentioned in-house code, leveraging Matplotlib [45] and seaborn [46]. The code to reproduce the analyses and visualization is available through GitHub (<https://github.com/viralemergence/zika-rnaseq-analysis>). Lastly, the figure labels were re-generated with Adobe Illustrator to increase readability, and the figures were exported from Illustrator to make high resolution files.

Results

Comparative growth curves

We first determined the susceptibility of two immortalized cell lines derived from the Egyptian rousette bat, *R. aegyptiacus* (R06E cells, Fig 1A), and the Jamaican fruit bat, *A. jamaicensis* (Aji cells, Fig 1B) to ZIKV isolates representing the African (ZIKV/MR766) or Asian-American (ZIKV/PRVABC59) lineages by performing multi-step growth curves (MOI=0.01). Our repeated measures ANOVA revealed significant main effects for all factors: cell line ($p<0.001$), virus strain ($p<0.001$), and

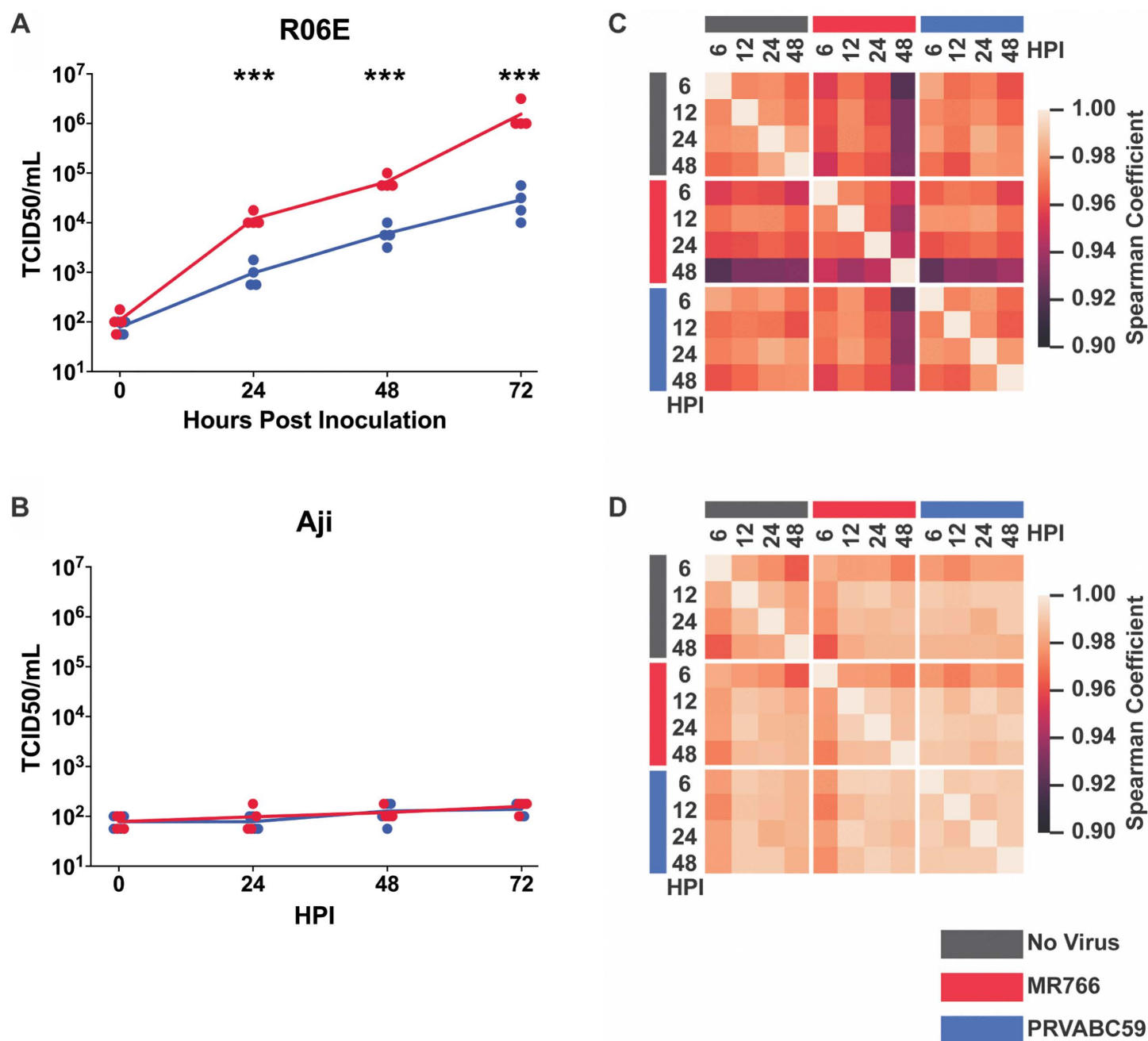


Fig 1. Comparative growth curves and normalized gene counts for Egyptian roussette bat (R06E) and Jamaican fruit bat (Aji) cell lines with African (MR766) and Asian-American (PRVABC59) ZIKV isolates. (A) Growth curves of ZIKV/MR766 and PRVABC59 isolates in R06E cells over 72 hours post-inoculation (HPI). (B) Aji cells did not support viral growth for either ZIKV isolate. Dots represent individual replicates; the lines represent medians. Repeated measures ANOVA revealed significant differences between virus strains in R06E cells at 24, 48, and 72 HPI (** $p < 0.0001$), but no significant differences in Aji cells at any time point. (C) Spearman correlation analysis of normalized gene counts in R06E cells showed high similarity among samples, with a minimum coefficient of 0.9201. (D) Aji samples exhibited even higher similarity, with a minimum Spearman coefficient of 0.9625. The colored bar legend in the bottom right corresponds to all panels of the figure.

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time ($p < 0.001$). We observed significant interactions for cell line $\times$ virus ($p < 0.001$), cell line $\times$ time ($p < 0.001$), virus $\times$ time ($p < 0.001$), and the three-way interaction cell line $\times$ virus $\times$ time ($p < 0.001$).

In Aji cells, we detected no significant differences between MR766 and PRVABC59 at any time point, with both viruses maintaining low, stable titers throughout the 72-hour period. In contrast, R06E cells supported robust viral replication with significant differences between virus strains emerging at 24 hours post-infection and persisting through 72 hours. Specifically, we found that MR766 achieved significantly higher titers than PRVABC59 in R06E cells at 24 HPI ($p < 0.0001$), 48 HPI ($p < 0.0001$), and 72 HPI ($p < 0.0001$). We observed no significant differences between virus strains at 0 HPI in either cell line, confirming equivalent initial inoculum levels. These results demonstrate that R06E cells are highly permissive to ZIKV replication for both strains, with MR766 showing superior growth kinetics, while Aji cells are non-permissive to both virus strains tested. We checked our transcriptomic data for expression of putative ZIKV receptors in the Aji cells and several were detected in the data ([S1 Table](#)).

Transcriptomic response to ZIKV infection *in vitro*

To assess the transcriptional responses of R06E and Aji cells to ZIKV infection, we performed total RNA sequencing on samples from both cell lines, either uninfected or infected with an African (ZIKV/MR766) or Asian-American (ZIKV/PRVABC59) lineage isolate at 6, 12, 24, and 48 HPI. After quality control and filtering (see Materials and Methods), gene counts were successfully obtained, normalized, and corrected for batch effects.

Spearman correlation analysis of gene counts revealed distinct patterns of similarity between treatment groups. In R06E cells, treatment groups were generally more similar to themselves than to other groups, though all samples remained highly correlated, with the lowest Spearman coefficient at 0.9201 ([Fig 1C](#)). Notably, within-group similarity decreased over time, even in untreated samples highlighting the importance of comparing the ZIKV experimental group against the negative control group at each time point rather than to 0 HPI. In contrast, Aji cells exhibited high correlation across all conditions, with the lowest Spearman coefficient at 0.9625 ([Fig 1D](#)), indicating minimal transcriptomic divergence between infected and uninfected states.

Principal Component Analysis (PCA) further supported these observations. In Aji cells, time was the dominant principal component, accounting for 98.41% and 98.44% of the variance in ZIKV/MR766- and ZIKV/PRVABC59-treated samples, respectively ([S1C](#), [S1D Fig](#)). Conversely, in R06E cells, time was not the primary driver of variability, contributing only 12.11% and 9.96% to the variance in MR766- and PRVABC59-treated samples, respectively ([S1A](#), [S1B Fig](#)).

Together, these findings suggest that R06E cells were successfully infected by ZIKV, as indicated by transcriptomic divergence following treatment. In contrast, the persistent similarity among Aji samples across time points and treatment conditions suggests that viral replication was effectively blocked in these cells, preventing downstream transcriptional changes associated with infection.

Gene set enrichment analysis reveals strong anti-viral response in Egyptian rousette cells to ZIKV infection. To characterize the global transcriptional response of R06E cells to ZIKV infection, we performed gene set enrichment analysis (GSEA) (see Materials and Methods). The most significant enrichment was observed at 6 HPI, with upregulated gene sets shared between both ZIKV/MR766 and PRVABC59 isolates. These included pathways involved in immune responses, such as “viral protein interaction with cytokine and cytokine receptor,” “malaria,” “rheumatoid arthritis,” and “toll-like receptor signaling pathway” ([Fig 2A](#), [2B](#)). Notably, no gene sets were significantly downregulated at this timepoint.

To assess strain-specific differences, we compared gene expression changes directly between ZIKV/MR766 and PRVABC59-infected R06E cells. The enrichment magnitude for all gene sets was markedly reduced ([Fig 2C](#)), though pathways associated with viral infection remained detectable. Among these, “viral protein interaction with cytokine and cytokine receptor” was the most consistently enriched across timepoints, suggesting that the African ZIKV isolate (ZIKV/MR766) elicits a stronger and earlier transcriptional response than the Asian-American ZIKV lineage isolate (ZIKV/PRVABC59).

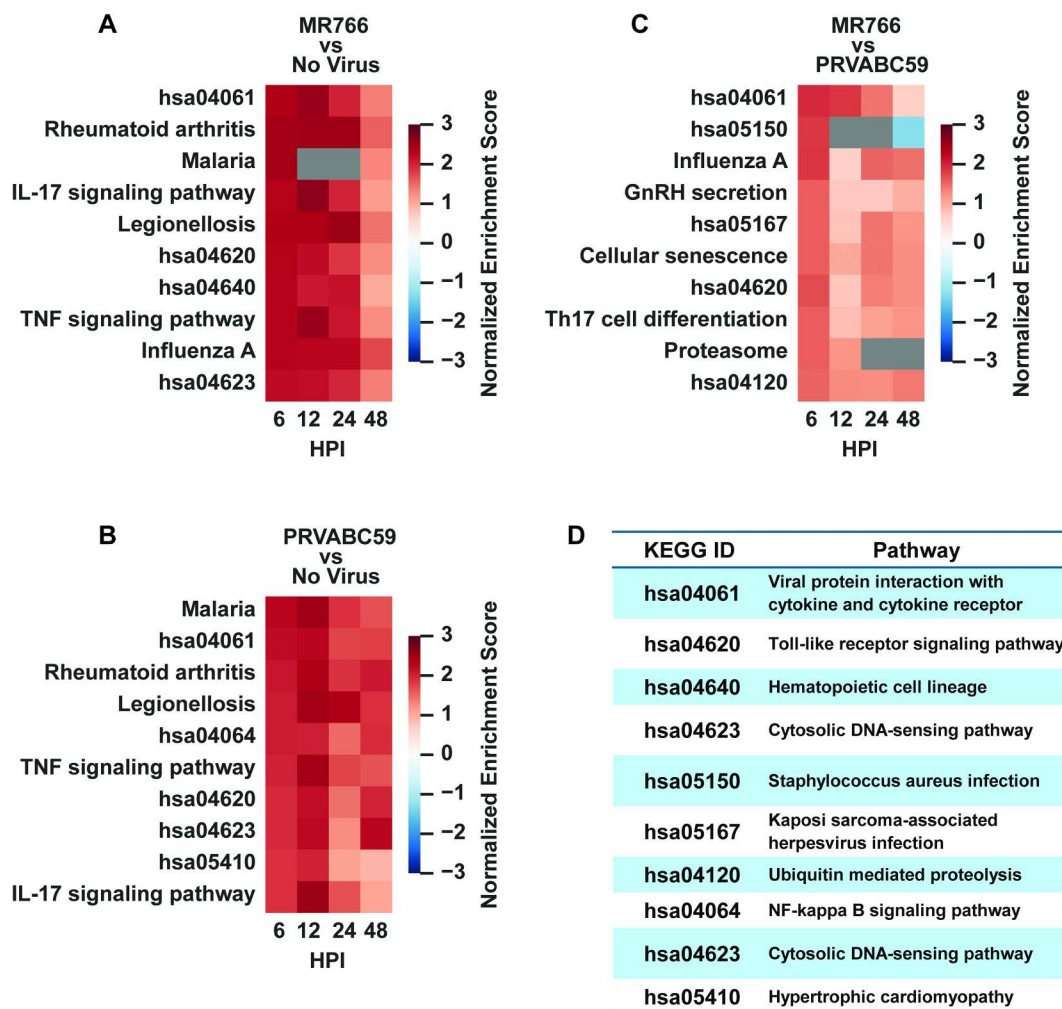


Fig 2. Gene set enrichment analysis (GSEA) of R06E cells following ZIKV infection. The top 10 upregulated gene sets at 6 HPI (FDR < 0.1) are shown, along with normalized enrichment scores (NES) across all timepoints. Grey boxes indicate timepoints where no NES was calculated due to a lack of upregulated genes in the gene set. (A) ZIKV/MR766-infected R06E cells showed enrichment of antiviral and pro-inflammatory gene sets. (B) ZIKV/PRVABC59-infected R06E cells displayed a similar enrichment pattern. (C) Direct comparison of ZIKV/MR766 vs. PRVABC59 in R06E cells revealed reduced enrichment of the same gene sets. (D) KEGG ID to associated pathway.

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To further validate our findings in Aji cells, we performed GSEA on these samples as well. However, nearly all enriched gene sets had false discovery rates (FDR) exceeding 0.1, rendering them statistically insignificant. The only significant gene sets for ZIKV/PRVABC59-infected Aji cells at 6 HPI included “ribosome,” “Parkinson disease,” and “valine, leucine, and isoleucine degradation,” which lack clear biological relevance to viral infection. Additionally, Spearman correlation analysis (Fig 1D) indicated that untreated Aji samples at 6 HPI were highly dissimilar from other Aji samples, suggesting a potential technical artifact influencing gene counts at this timepoint. We acknowledge that limited replication in some Aji cell timepoints necessitates cautious interpretation of transcriptional non-responsiveness, though this conclusion is supported by multiple independent lines of evidence including viral growth kinetics and global transcriptomic patterns.

Overall, these results reinforce that R06E cells support ZIKV replication and mount a robust immune response, while Aji cells do not exhibit a clear transcriptional signature of infection. Consequently, Aji samples were excluded from subsequent analyses.

Gene ontology enrichment analysis (GOEA) corroborates GSEA results. The GSEA results generally correspond to what we would expect based on ZIKV literature, but we recognize using human gene sets for the GSEA could create some species-specific bias or miss species-specific effects when considering responses in bats. To corroborate our findings with an orthogonal approach, we performed gene ontology enrichment analysis (GOEA) using differentially expressed genes (DEGs) (see Materials and Methods). Many enriched GO terms were redundant, so we grouped them using GO slim categories and selected the most significant term within each group for clarity (S2 Fig, S3 Table).

The top 10 statistically significant, enriched GO terms for each viral contrast are shown in Fig 3. ZIKV/MR766-infected R06E cells exhibited strong enrichment for immune-related processes, including “defense response to symbiont,” “immune response,” and “cell surface signaling pathway” (Fig 3A). ZIKV/PRVABC59-infected cells displayed similar enrichment patterns, with top terms including “defense response,” “cytokine-mediated signaling pathway,” and “neutrophil chemotaxis” (Fig 3B). These results align with the previously identified enriched gene sets, such as “Influenza A” and “viral protein

interaction with cytokine and cytokine receptor” (Fig 2A, 2B). When directly comparing R06E transcriptional response to infection with ZIKV/MR766 versus ZIKV/PRVABC59, GOEA revealed significant differences in terms such as “defense response to virus” and “type I interferon-mediated signaling pathway” (Fig 3C). However, the statistical significance and gene support for these terms were reduced, mirroring the decrease in normalized enrichment scores observed in GSEA (Fig 2C). Together, GOEA and GSEA results indicate that both ZIKV isolates induce similar immune responses in R06E cells, with ZIKV/MR766 eliciting a stronger antiviral and pro-inflammatory response.

DEG clustering and GOEA suggest subtle differences in ZIKV/MR766 and ZIKV/PRVABC59-induced responses. The previous analyses indicate that ZIKV/MR766 and PRVABC59 elicit similar antiviral and pro-inflammatory responses in R06E cells. However, GSEA relies on predefined gene sets, and our GOEA did not distinguish between up- and downregulated genes, though most DEGs were upregulated. To further explore transcriptional responses, we clustered DEGs based on similar expression profiles across timepoints and analyzed their functional enrichment.

Since previous analyses showed strong similarity between ZIKV/MR766- and PRVABC59-infected R06E cells, we focused on this comparison to highlight isolate-specific differences in the transcriptional response. DEG expression clustering identified three major groups. Group 10 exhibited a general increase in expression across all timepoints (Fig 4A) and was enriched for antiviral GO terms such as “defense response to virus” and “type I interferon-mediated signaling pathway (GO:0060337)” (Fig 4B), driven by interferon-stimulated genes including *OAS3*, *MX2*, *IFIT3*, *IFI6*, and *DDX58*. Additionally, ZIKV/MR766 elicited a stronger response at 6 HPI than PRVABC59, consistent with GSEA findings (Fig 2C).

In contrast, Groups 5 and 8 showed more distinct expression patterns, with ZIKV/MR766 eliciting stronger responses at 6 and 48 HPI, but similar levels at 12 and 24 HPI (Fig 4C, 4E). These groups were enriched for less intuitive GO terms, including “GTPase activator activity” (Fig 4D) and “transmembrane receptor protein tyrosine kinase activity (GO:0004714)” (Fig 4F). Many flaviviruses, including ZIKV, enter host cells through clathrin-mediated endocytosis, a process that depends heavily on small GTPases [47] and tyrosine kinase receptors to facilitate viral entry, replication, and egress [48]. Consequently, while the exact roles of groups 5 and 8 in viral response remain unclear, these findings suggest additional biological processes may contribute to ZIKV infection dynamics.

CCL20, CCL5, CXCL8, and IL6 may play a role in the differential response between ZIKV/MR766 and PRVABC59 infected R06E cells. Given that the “viral protein interaction with cytokine and cytokine receptor” gene set was more enriched than any other in the ZIKV/MR766 vs PRVABC59 contrast (Fig 2C), we further examined its gene composition, focusing on upregulated genes. GSEA at 6 HPI revealed that the enrichment was primarily driven by *CCL20*, *CCL5*, *CXCL8*, and *IL6*, which ranked among the most upregulated genes in the set (Fig 5A). Heatmap analysis and manual

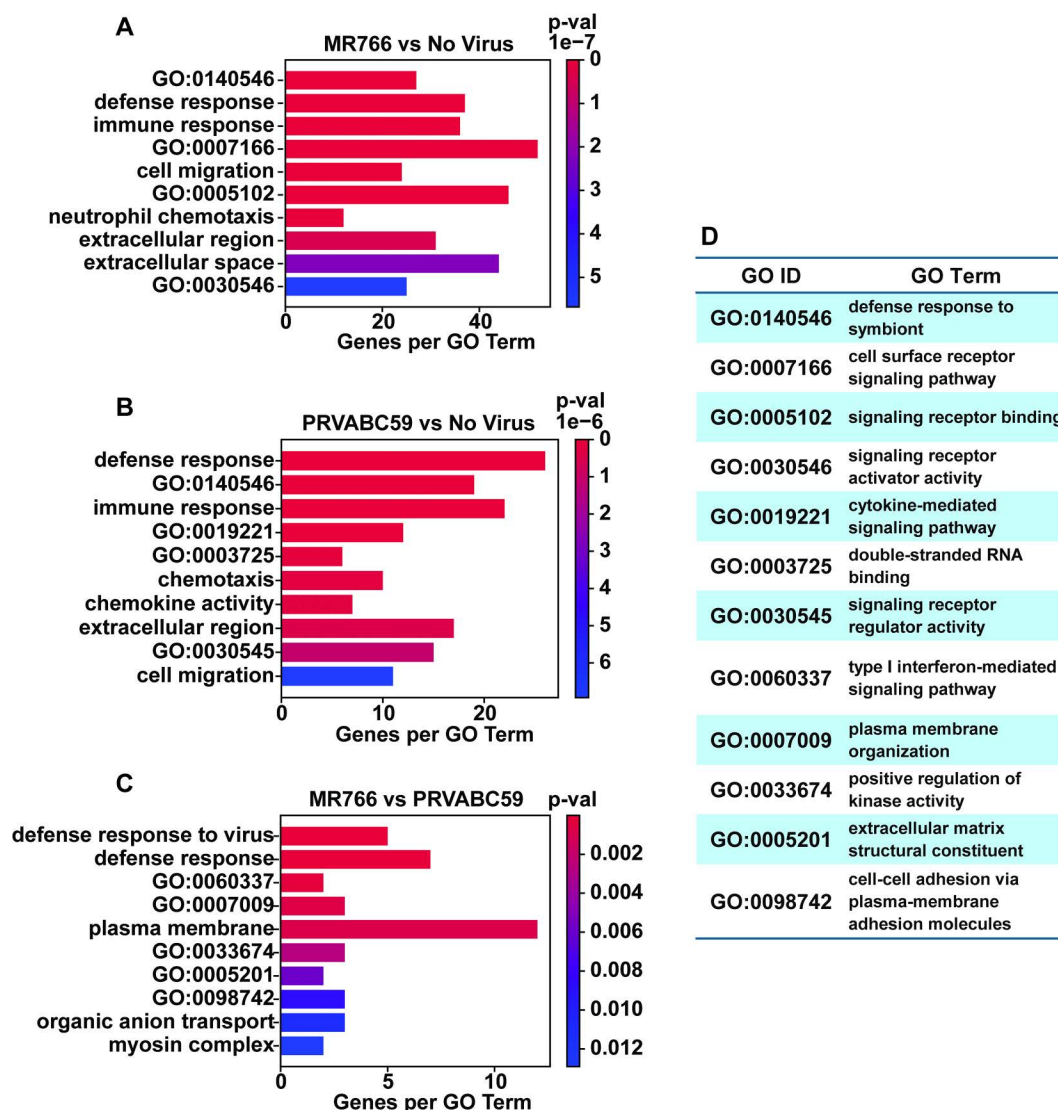


Fig 3. Gene ontology enrichment analysis (GOEA) of differentially expressed genes (DEGs) in ZIKV-infected R06E cells. GOEA was performed on DEGs from each viral contrast to identify enriched GO terms. Terms were grouped by GO slim categories, with the most significant term representing each group. The top 10 enriched GO terms are shown, color-coded by unadjusted p-values. (A) ZIKV/MR766-infected R06E cells showed strong enrichment for antiviral pathways. (B) ZIKV/PRVABC59-infected cells displayed a similar enrichment pattern. (C) Direct comparison of ZIKV/MR766 vs. PRVABC59 revealed reduced enrichment, with lower significance and fewer genes contributing to each GO term. (D) GO identification numbers to functional categories.

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inspection confirmed that *CCL20*, *CCL5*, *CXCL8*, and *IL6* exhibited some of the highest log₂ fold changes within the set at 6 HPI, although they do generally decrease over time (Fig 5B).

To better understand the relationship between these genes, we used the STRING database [43] to generate a protein-protein interaction (PPI) network of the analyzed genes in the set (Fig 5C; see Materials and Methods). We observed that *CCL5*, *CXCL8*, and *IL6* emerged as hub genes having the highest number of interactions with other proteins (16 interactions) in the network (average of 10.63 interactions). Hub genes likely perform a critical role in modulating the entire network or performing other biological processes.

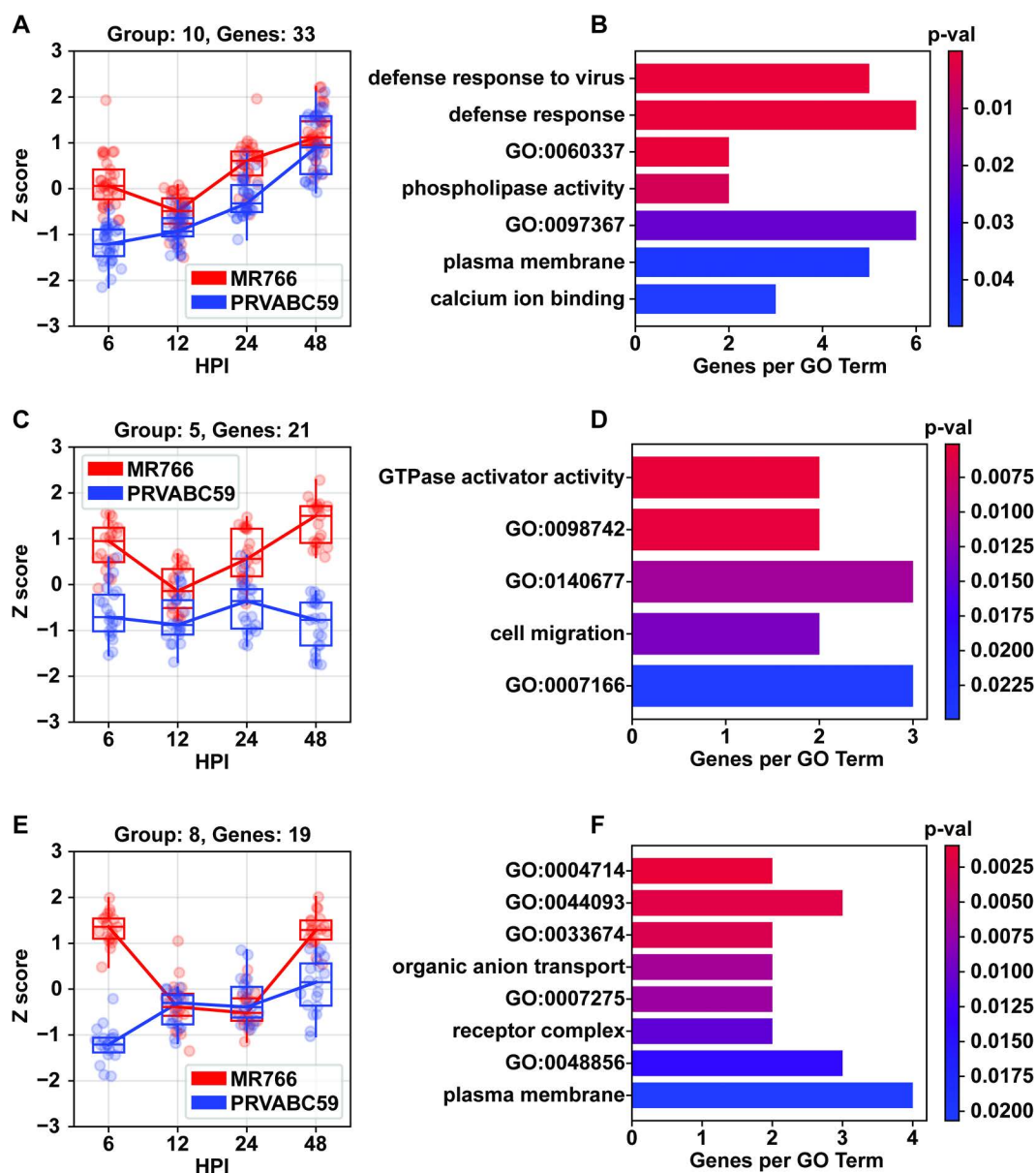


Fig 4. Clustering of differentially expressed genes (DEGs) in R06E cells infected with African (MR766) and Asian-American (PRVABC59) ZIKV isolates. Boxes represent quartiles and median values, while dots indicate mean relative abundance per gene. (A) Group 10 genes increased in expression over time, with larger divergence at 6 HPI. (B) GOEA revealed enrichment for defense response terms. (C, E) Groups 5 and 8 showed increased expression at 6 and 48 HPI in MR766-infected cells. (D, F) However, some enriched GO terms were not clearly linked to antiviral activity. For bars labelled with GO IDs, see S3 Table for full GO terms.

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Discussion

This study investigated the *in vitro* susceptibility and immune responses of immortalized cell lines derived from two frugivorous bat species following inoculation with African and Asian-American ZIKV lineages. We observed striking inter-species differences in susceptibility with R06E cells (*R. aegyptiacus*) supporting robust viral replication (Fig 1A) while Aji cells (*A. jamaicensis*) appeared resistant to infection (Fig 1B). Infection with ZIKV in R06E cells induces a strong, early,

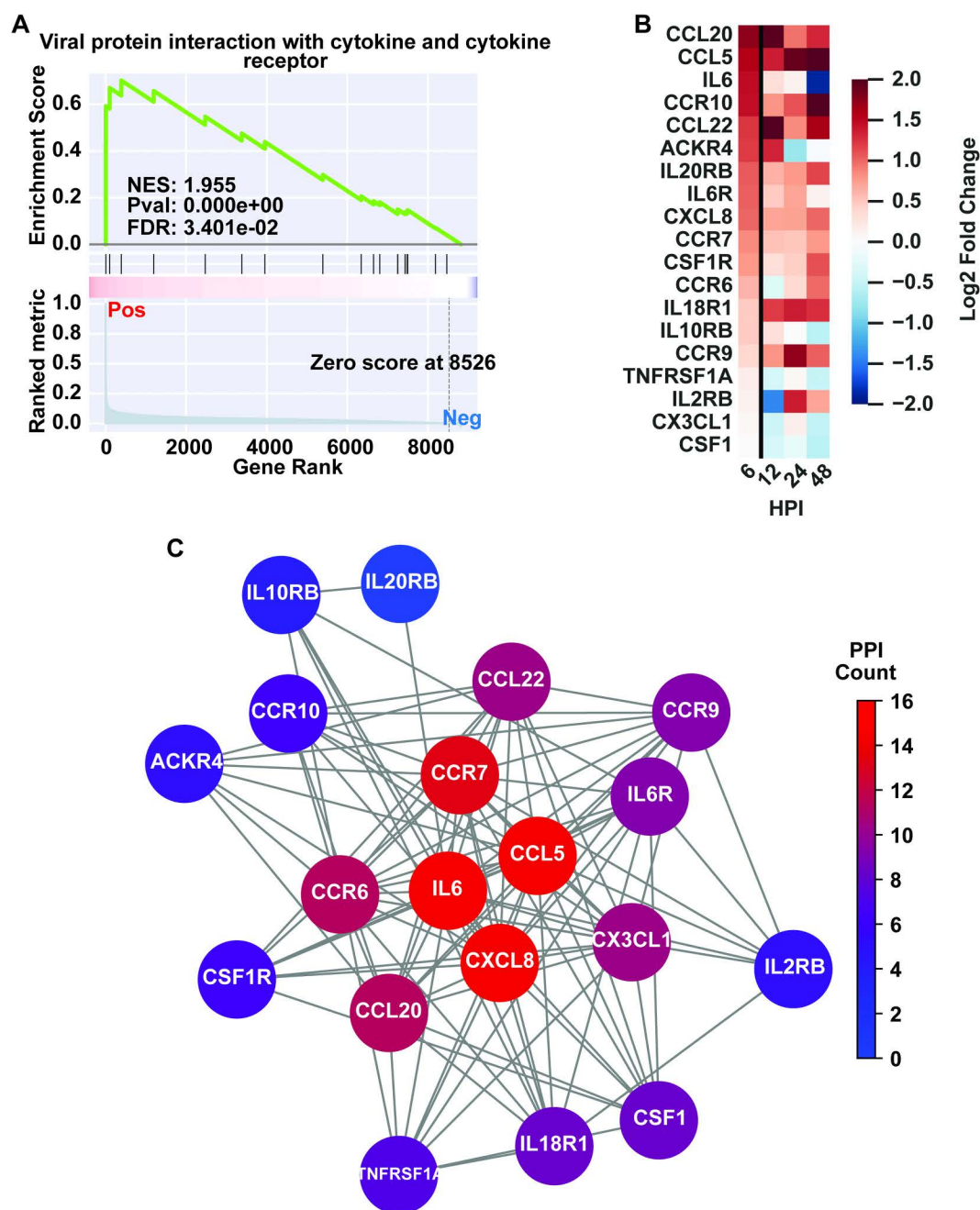


Fig 5. Differential gene expression and network analysis of cytokine-related genes in ZIKV/MR766 vs. PRVABC59. (A) Gene set enrichment analysis (GSEA) for “Viral protein interaction with cytokine and cytokine receptor” in ZIKV/MR766 vs. PRVABC59 (upregulated DEGs). The running enrichment score (ES) peaks early, driven primarily by *CCL20*, *CCL5*, *CXCL8*, and *IL6*, with a normalized enrichment score (NES) of 1.955—the highest in this contrast. (B) Log₂ fold changes of set genes show upregulation across HPI, with the leading genes among the most highly upregulated. (C) Protein-protein interaction (PPI) network from the STRING database, highlighting *CCL5*, *CXCL8*, and *IL6* as key hub genes with extensive connectivity.

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and lineage-specific antiviral response (Figs 2 and 3) with upregulation of several inflammatory cytokines including CCL5, CXCL8, IL6, and CCL20 (Fig 5). These findings raise new hypotheses about host-specific factors governing ZIKV susceptibility. In addition, our results provide insights into the range of cellular responses to infection for laboratory-derived bat cell lines.

The absence of ZIKV replication or transcriptional immune response in Aji cells could be governed by viral attachment/entry factors (i.e., reversible adsorption, absence of cellular entry receptors and/or attachment factors) or post-entry events (i.e., unsuccessful viral uncoating after endocytosis). Transcriptomic data revealed that our Aji cells do express several genes associated with putative ZIKV receptors or attachment factors and suggests that attachment/entry factors alone do not explain their apparent resistance (S1 Table). Rather, a post-attachment/entry mechanism potentially affecting viral uncoating, replication, or early host sensing may contribute to the observed lack of transcriptional immune activation and limited permissiveness (Fig 2D). These data contrast with *in vivo* findings reporting ZIKV antigen detection in mononuclear cells of experimentally challenged *A. jamaicensis* bats, but it is noteworthy that neither *in vivo* study described productive ZIKV infection suggesting a post-entry resistance mechanisms [27]. It is possible that our immortalized, kidney derived Aji cells with adherent, fibroblast morphology are not a permissive cell type; however, the R06E cells, derived from “the body of an Egyptian rousette bat fetus” are also adherent with fibroblast morphology and do support ZIKV replication (Fig 1A). Because these experiments rely on immortalized cell lines derived from different tissues and developmental stages, the observed differences should be interpreted as cellular phenotypes rather than being representative of species-wide susceptibility or resistance. While our findings are consistent with limited cellular permissiveness observed *in vivo* and the results of biosurveillance efforts with *Artibeus* spp. bats [23,24,26,25], these data should be interpreted as reflecting cell-intrinsic barriers that limit productive infection rather than generalized species-level resistance phenotype. For example, Reagan *et al.* (1955) reported neurologic disease (described as “nervous symptoms”) in 16 of 20 New World cave bats (*Myotis lucifugus*) inoculated with ZIKV, suggesting that other bat species in the Americas may be susceptible to infection with ZIKV [49].

R06E cells supported productive replication of both African (ZIKV/MR766) and Asian-American (ZIKV/PRVABC59) lineage ZIKV isolates (Fig 1A), indicating that *R. aegyptiacus* cells are permissive to ZIKV infection. Because productive replication requires successful evasion of host antiviral defenses, viral replication in host cells represents an important, though not definitive, criterion for identifying potential contributions to sylvatic transmission cycles. Our data with R06E cells aligns with field surveillance efforts reporting detection of ZIKV subgenomic flaviviral RNA (sfRNA) in free-ranging *R. aegyptiacus* [4] and low-levels of viremia in experimentally challenged bats [18].

Despite efficient ZIKV replication in R06E cells (Fig 1A), the associated transcriptional immune response did not resemble the attenuated or tightly regulated inflammatory profiles that have been described for well-adapted bat-virus relationships in other systems (Fig 6). For example, *R. aegyptiacus* are considered the primary reservoir for the highly pathogenic Marburg virus (MARV), characterized by a controlled inflammatory response to MARV infection [50]. *In vitro* experimental work with *R. aegyptiacus* dendritic cells showed a strong upregulation of type I IFN-related genes with proinflammatory responses downregulated upon infection [51]. *In vivo* experiments with *R. aegyptiacus* further demonstrated canonical antiviral responses with minimal and organ-specific proinflammatory signaling, notably lacking upregulation of *IL6* and *CCL8*, which are associated with severe disease in humans and nonhuman primates [52,53]. The notion of immune tolerance during viral infection through controlled inflammatory response has been widely suggested across several bat-virus systems [54,55], though the extent of this mechanism for tolerating viral infection in bats remains unresolved [54].

Although we hypothesized that *R. aegyptiacus* might exhibit a similarly immune tolerant profile following ZIKV infection, our findings instead reveal a more complex and context-dependent pattern. Both African (ZIKV/MR766) and Asian-American (ZIKV/PRVABC59) isolates triggered robust antiviral and pro-inflammatory responses in R06E cells. Gene set enrichment analysis (GSEA) of R06E cells revealed upregulation of immune-related pathways at 6 HPI, including “viral

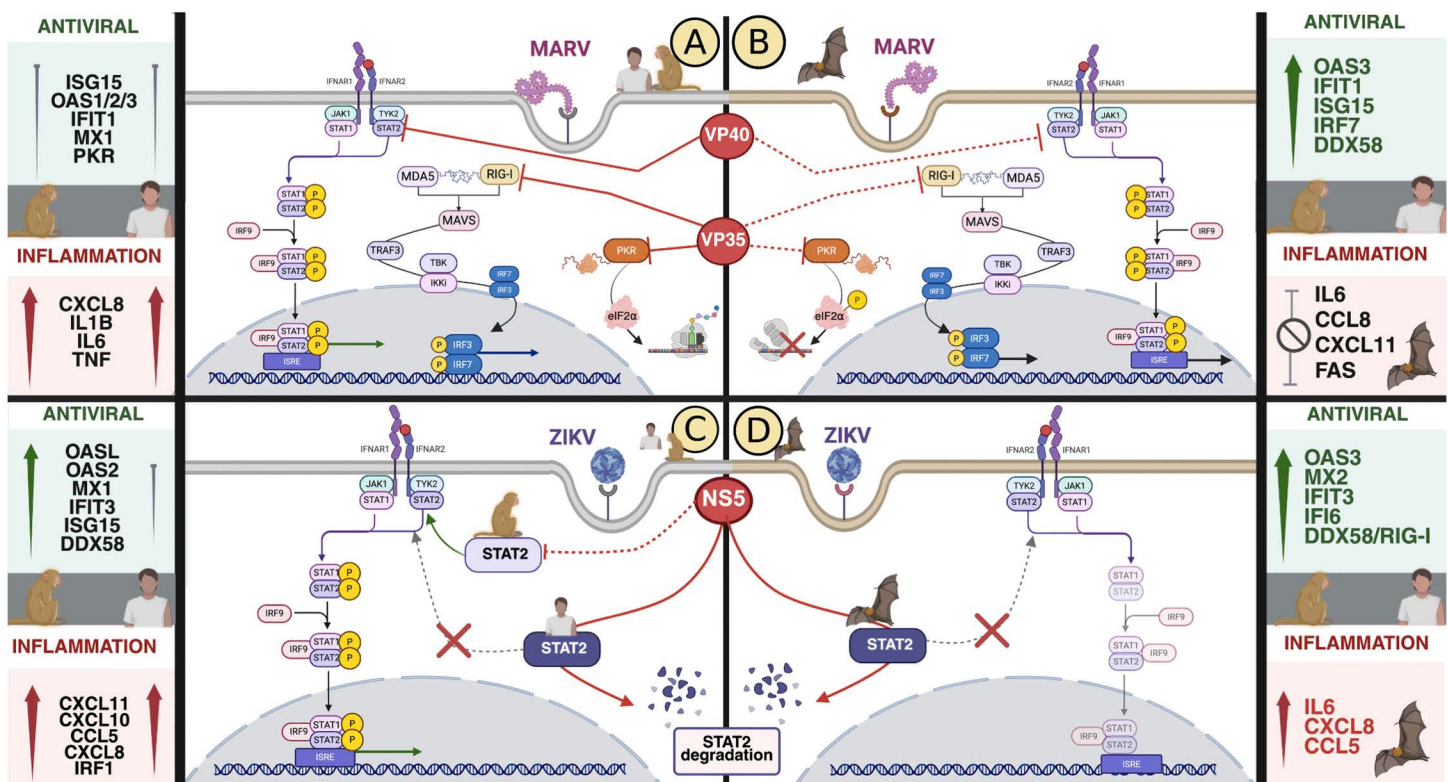


Fig 6. Hypothesized mechanisms governing host-virus interactions during in vitro infection of *Rousettus aegyptiacus* cells with Marburg (MARV) and Zika (ZIKV). ((A) MARV infection in humans and non-human primates, (B) MARV infection in *Rousettus aegyptiacus*, (C) Zika virus infection in humans and non-human primates, and (D) Zika virus infection in *Rousettus aegyptiacus*. Created in BioRender by Fagre, A. (<https://BioRender.com/04efbsj>) and is licensed under CC BY 4.0.

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protein interaction with cytokine and cytokine receptor”, “toll-like receptor signaling,” and “rheumatoid arthritis” (Fig 2A, 2B). These pathways are commonly associated with innate immune activation against viral infections, particularly those driven by interferon-stimulated genes (ISGs).

Notably, R06E cells exhibited robust induction of ISGs such as *OAS3*, *MX2*, *IFIT3*, *IFI6*, and *DDX58*, suggesting partial or no degradation of *R. aegyptiacus* STAT2 by ZIKV NS5 (Fig 6). These findings contrast with prior reports of efficient degradation of *Pteropus alecto* bat STAT2 by ZIKV [47]. Interestingly, the magnitude of immune response in R06E cells was stronger for the African lineage (ZIKV/MR766) than the Asian-American lineage (ZIKV/PRVABC59) (Fig 2C). Taken together, these observations warranted further functional interpretation through Gene Ontology enrichment analysis (GOEA) to better contextualize the transcriptional changes.

GOEA corroborated our GSEA findings, with R06E cells displaying strong enrichment for immune-related terms (Fig 3A, 3B). Among the most significant were “defense response to symbiont,” “immune response,” and “cytokine-mediated signaling”, reinforcing the notion that ZIKV infection in R06E cells activates innate immune pathways. Differentially expressed gene (DEG) clustering revealed three major transcriptional groups in R06E cells. Group 10 genes, including *OAS3*, *MX2*, *IFIT3*, *IFI6*, and *DDX58*, exhibited consistent upregulation across timepoints, confirming their role in ZIKV-driven antiviral defense (Fig 4A, 4B). Notably, ZIKV/MR766 induced a stronger response at 6 HPI than ZIKV/PRVABC59, consistent with our GSEA and GOEA findings. However, Groups 5 and 8 contained genes with unique expression patterns, being more strongly induced at 6 and 48 HPI in ZIKV/MR766 infections (Fig 4C, 4E). These groups were enriched

for “GTPase activator activity” and “transmembrane receptor protein tyrosine kinase activity” (Fig 4D, 4F), both of which are involved in viral entry, trafficking, and immune signaling. The exact role of these pathways in ZIKV infection remains unclear, but their enrichment suggests potential alternative mechanisms regulating host susceptibility and immune activation. Given the strong enrichment of “viral protein interaction with cytokine and cytokine receptor” in MR766-infected R06E cells, we examined specific genes within this set. GSEA at 6 HPI identified *CCL20*, *CCL5*, *CXCL8*, and *IL6* as among the top upregulated genes (Fig 5A and 5B). Further, protein-protein interaction (PPI) network analysis identified *CCL5*, *CXCL8*, and *IL6* as major hub genes, suggesting they play central roles in the immune response to ZIKV/MR766 infection (Fig 5C).

The observed transcriptional responses in R06E cells are consistent with literature on ZIKV infection in other mammals, namely humans and primates, rather than the immune tolerant phenotype observed in R06E cells following infection with MARV [53] (Fig 6). Previous findings from Viet et al. show that flavivirus NS5 proteins—particularly from ZIKV—partially antagonize STAT2-dependent signaling, including in various bat taxa [56]. While our transcriptomic analysis of R06E cells revealed robust upregulation of ISGs, including *OAS3*, *MX2*, and *DDX58*, we found that R06E cells supported robust replication of ZIKV. It is possible that degradation of STAT2 in ZIKV-infected R06E cells did dampen interferon signaling, and ISG transcription was driven by alternative STAT-independent pathways (e.g., IRF3/IRF7 activation by RIG-I). Though our current dataset does not allow for mechanistic dissection of these signaling routes, the consistent upregulation of ISGs despite evidence of STAT2 degradation by ZIKV NS5 warrants further investigation into non-canonical interferon signaling in this species.

Conclusions

In conclusion, our comparative *in vitro* analyses reveal striking interspecies differences in ZIKV susceptibility and immune activation between *R. aegyptiacus* and *A. jamaicensis* cell lines. While *R. aegyptiacus* cells supported productive replication of both African and Asian-American ZIKV lineages and mounted a robust antiviral and proinflammatory transcriptional response, *A. jamaicensis* cells exhibited no evidence of productive infection or immune activation, consistent with post-entry resistance mechanisms. The inflammatory profile in *R. aegyptiacus* cells more closely resembled that of non-reservoir mammalian hosts than the immune-tolerant responses observed in established bat-virus reservoir systems, suggesting that ZIKV is unlikely to be a well-adapted virus in this species. However, the persistence of ISG upregulation despite reported STAT2 antagonism hints at alternative, IFN-independent antiviral signaling routes that may shape the host response.

While *in vivo* infection studies are ultimately required to determine host competence, viral infection tolerance, and potential contributions to onward transmission via mosquito vector, such experiments in bats are logistically challenging. In this context, *in vitro* systems represent a critical and widely accepted first step for assessing cellular permissiveness and early host-virus interactions, and for generating mechanistic hypotheses that can be evaluated *in vivo*. The replication dynamics and immune transcriptional profiles observed here provide a foundation for future experimental infection studies by helping to frame biologically informed questions regarding host responses to ZIKV, rather than serving as definitive evidence of reservoir competence. Collectively, our findings reveal clear differences in cellular permissiveness and innate immune activation following ZIKV exposure in bat-derived, experimental systems. These results complement field and *in vivo* observations and underscore the value of *in vitro* models for dissecting early determinants of ZIKV host range.

Geographic expansion of pathogens often drives novel host-virus interactions with unpredictable immune responses, particularly in wildlife systems which pose significant challenges for researchers when assessing zoonotic risk in dynamic conditions such as rapid spread of ZIKV in the Americas. Thus, characterizing the cellular permissiveness and transcriptional responses using *in vitro* systems contributes essential data to inform a broader risk assessment framework for pathogens of public health importance.

Supporting information

S1 Fig. Principal Component Analysis (PCA) of normalized gene expression in R06E and Aji cell lines with MR766 and PRVABC59 treatment. Each PCA contains the data for both negative and treated samples, represented as individual dots. The samples are colored by the hours post infection (HPI) at which they were collected. (A) PCA for R06E cells treated with MR766 reveals the first principal component (PC1) does not coincide with time, whereas the second component (PC2) does roughly. (B) Likewise, PCA for R06E cells treated with PRVABC59 shows a similar pattern. Together, these results suggest that while time does affect changes in expression for R06E cells, it is not the primary driver, only accounting for 12.11% and 9.96% of variation, respectively. (C) Inversely, PCA for Aji cells treated with MR766 shows that PC1 does roughly coincide with time, as does (D) Aji cells treated with PRVABC59, with these components accounting for 98.41% and 98.44% of variation, respectively. Together, this suggests that Aji cells may not have been infected and almost all change in expression is reflects the passage of time.

(DOCX)

S2 Fig. Gene Ontology (GO) chart explaining grouping and selection of GO terms. Shown is an example GO chart generated with QuickGO, identifying the parental GO terms above “defense response to virus”. In a standard GO enrichment analysis (GOEA), many of the parental GO terms are identified as enriched, making results lengthy and redundant. To simplify results, enriched GO terms were mapped to a subset of manually curated GO terms called GO Slim terms. In this figure, that would mean all child GO terms under the GO Slim term “biological process involved in interspecies interaction” (boxed in red) would be grouped together (e.g., GO:0051707, GO:0098542, GO:0009615, GO:0051607). However, these GO Slim terms are very broad and less informative. Consequently, we chose to select the most statistically significant GO term in the group to represent the entire group (i.e., “defense response to virus” boxed in green). This dramatically reduced the number of GO terms to report and provided clearer insight into the function of differentially expressed genes.

(DOCX)

S1 Table. Batch corrected counts for genes identified as putative receptors for ZIKV that were detected and annotated in the Aji cell line.

(CSV)

S2 Table. Experimental replicate counts. The number of experimental replicates is provided with the first column containing metadata about the sample type and the second column listing the replicate count. The samples are described with the format of {cell line}-{virus treatment}-{timepoint}. E.g. R06E-PRV-24.0 denotes the R06E cell line infected with PRVABC59 and collected at 24 hours post infection had 3 experimental replicates.

(DOCX)

S3 Table. Gene ontology enrichment analysis (GOEA) results. The table file contains the entire comprehensive results for GOEA. There are 6 tabs including “MR_vs_No_Virus”, “PRV_vs_No_Virus”, and “MR_vs_PRV” corresponding to the analyses done in Fig 3. The tabs “Group_10”, “Group_5”, and “Group_8” correspond to the analyses done in Fig 4.

(XLSX)

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