Shared transcriptomic responses of *Aedes aegypti* to arboviral infections : example of dengue and Rift Valley fever viruses

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Research

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Abstract

Background

Arthropod borne virus infections cause several emerging and resurgent infectious diseases. Among the diseases caused by arboviruses, dengue and Rift Valley fever are responsible for a high rate of severe human diseases worldwide. Understanding the effects of viral infection on gene expression in the mosquito is crucial to the development of early diagnostic tools and may enable researchers and policy makers to better anticipate outbreaks in the next future.

Methods

Here we investigate the alterations in gene expression across the entire *Aedes aegypti* genome during infection with DENV and RVF over time.

Results

We describe several up-regulated genes that share a similar expression profile during infection with both viruses at early and late phases of infection. Family B and D clip-domain serine proteases (CLIP) are clearly overrepresented as well as C-type lectins and transferrin.

Conclusions

Our results provide an extensive amount of data highlighting viral gene targets in the mosquito during infection. This data may also be used to develop broad-spectrum anti-viral diagnostic tools based on mosquitoes rather than the mammalian hosts and would help to predict and manage the emergence of outbreaks.

Background

In the past decade, there has been a worldwide emergence of arboviruses-related diseases such as zika, chikungunya, dengue, West Nile and Rift Valley fever (RVF) leading to a global health burden with important socioeconomic impacts. These diseases induce similar clinical manifestations at early stage of infection in their hosts and are mostly transmitted by the same mosquito species.

Rift Valley fever virus (RVFV) is a category A priority zoonotic pathogen due to its potential to cause severe economic distress and major health issues (mass abortions and neonatal mortality in ruminants, human deaths) following infection by direct contact with infected animals (tissues, aerosols) or through the bites of infected mosquitoes. RVFV is widespread in Sub-Saharan Africa and has expanded its geographic range to Egypt including the River Nile Delta, the Arabian Peninsula and in the Indian Ocean zone (the Comoros archipelago and Madagascar) [1-4].

Dengue caused by dengue virus (DENV) belonging to the family Flaviviridae, genus Flavivirus, species *dengue virus* is endemic in more than 100 countries in southeast Asia, the Americas, the western Pacific, Africa and the eastern Mediterranean regions [5].

The ongoing outbreaks of RVF occurring in the eastern Horn of Africa (Kenya, Uganda, Rwanda) and in Mayotte, a French island part of the Comoros archipelago [6,7] as well as outbreaks of dengue in the Indian Ocean area (La Reunion, Mayotte and Seychelles islands) [8,9] underline the need of a better control of both diseases by implementing early warning systems resulting from interactions between people, mosquitoes, arboviruses, and environmental factors. They may possibly help in anticipating or preventing the spread of huge outbreaks through adequate control measures. Efforts have been made to better understand the mechanisms of pathogenesis of these viruses including pathways essential for replication and to develop innovating and appropriate diagnostic tools, therapeutics and vaccines. Current diagnostic methods mainly developed for the detection of specific viral molecular signatures in the host compartment by molecular based techniques [10,11] were adapted to trapped arthropods [12-15] with limitations such as a (i) the need of high viral load being present in the vector at the time of the trapping, (ii) the time window in which the competent vectors exhibit a sufficient viral load, (iii) the specificity of the detection of one pathogen rather than having a larger detection system able to detect several arboviral infections at the same time. Applicability of other assays such as the Lawrence Livermore Microbial Detection Array (LLMDA) revealed the presence of mosquito-borne viruses and insect-specific viruses in field-collected mosquitoes with similar limitations [16]. Recently, in 2018, Fukutani and colleagues [17-18] proposed an innovative approach, based on the transcriptomic response of the vectors to various arboviruses. In short, they listed a group of co-regulated genes whose expression levels significantly changed in vectors infected by different viruses.

Indeed, one could expect that native immunity related genes could be expressed in a non pathogen-specific manner in response to infection. A set of 110 co-regulated genes, with a subset of 4 genes whose expression values according to their methods allowed the classification of vectors as infected/non infected. This approach is paving the way to surveillance methods that allow for a non-specific detection of arboviral diseases in vectors responsible of their transmission. Following this approach, our study was designed to search for up-regulated genes in *Aedes aegypti* cells lines in response to two major arboviruses infections, dengue and Rift Valley fever viruses infections by RNA sequencing.

Therefore, getting an early diagnostic tool involving a set of markers able to detect early signs of viral infection in the vector component rather than waiting for the clinical signs to exacerbate in the host component of the epidemiological cycle is an alternative way to better anticipate epizootics/epidemics simultaneously.

Methods

Cell culture, virus growth and viral infections

The stock of the Smithburn RVFV strain (OBP, Onderstepoort Biological Products, South Africa) and the stock of serotype 2 human DENV supplied by Dr Jaffar-Bandjee for research purposes (CHU La Réunion) were produced on African green monkey kidney Vero cells using DMEM (Gibco, France) containing 10% foetal bovine serum (FBS) (Gibco Life Technologies, France), 2mM L-Glutamine (Gibco Life Technologies, France). Virus-containing medium was harvested when the cytopathic effect (CPE) exceeded 75 %, and the viral infectivity titre was determined by limiting dilution [19].

The CCL-125 *Aedes aegypti* derived cell line Aag2 (Pasteur Institute, Paris, France) [20] was used for infection studies. The cells were grown at 28°C in Schneider's drosophila medium (Gibco Life Technologies, France) supplemented with 10% FBS (Gibco Life Technologies, France), 1% L-Glutamine 1mM, 1% Penicillin-Streptomycin (Gibco Life Technologies, France). Cells were infected at a multiplicity of infection (MOI) of 0.1 for RVFV and at a MOI of 1 for DENV. Viruses were allowed to propagate for 24 hours and 6 days to assess early and late genes expression. Three controls were included, non infected Aag2 cells to assess the expression of genes related to cellular functions (Mock A), RVFV (MOI of 0.1) and DENV (MOI of 1) infected and UV inactivated cells to assess the expression of activated genes following the viral entry into the cells (Mock B), and non-infected Aag2 cells submitted to a heat shock through an incubation step at 37°C for 30 minutes to assess the expression of genes involved in cellular oxidative stress (Mock C). Supernatants were removed and spun down, cells were harvested and stored at -80°C until use. All experimental infections were performed under biosafety level 3 (BSL-3) conditions. The experiment was undertaken independently in triplicates.

RNA extraction

Total RNA was isolated from DENV serotype 2, RVFV infected *Ae. aegypti* and the three controls (Mock A, B and C) supernatants on days 1 and 7 using the NucleoSpin RNA virus kit (Macherey-Nagel, France) according to the manufacturer's instructions except that the lysis buffer RAV1 was supplemented with linear acrylamide (Ambion, Life technologies, France) at a concentration of 5 mg/ml and Proteinase K (20 mg/ml) instead of the RNA carrier provided in the kit. The eluted RNA was submitted to a Turbo Dnase digestion (Ambion, Life technologies, France) (2 units/ml) for 30 min at 37°C, followed by inactivation and clean up steps performed with the RNeasy MinElute Cleanup kit (Qiagen, France) and stored at -80ºC until used. The quality of the isolated total RNA from each sample was checked using the Nano RNA chips in 2100 Bioanalyzer (Agilent, France) and the quantitation was performed using the NanoDrop 2000 (ThermoFisher Scientific, France). The RNA samples with RNA Integrity Number (RIN) ≥8 were selected for library preparation.

DENV and RVFV viral infections were confirmed by previously described RT-qPCR [10,21]. Briefly, for each sample, qPCR reactions were performed in triplicate using the AgPath One Step RT-PCR 2X Mix (Ambion, Life Technologies, Santa Clara, USA) in an Mx 3005P QPCR System ™ (Stratagene, USA).

Library construction for RNA sequencing

Libraries were constructed using the Truseq stranded mRNA sample prep kit (Illumina, ref. RS-122-2101) according to the manufacturer instructions. Briefly, poly-A RNAs were purified using oligo-d(T) magnetic beads. The poly-A+ RNAs were fragmented and reverse transcribed using random hexamers, Super Script II (Life Technologies, ref. 18064-014) and Actinomycin D. During the second strand generation step, dUTP substitued dTTP. This prevents the second strand to be used as a matrix during the final PCR amplification.
Double stranded cDNAs were adenylated at their 3' ends before ligation was performed using Illumina's indexed adapters. Ligated cDNAs were amplified following 15 cycles PCR and PCR products were purified using AMPure XP Beads (Beckman Coulter Genomics, ref.A63881). Libraries were validated using a Fragment Analyzer (Agilent) and quantified using the KAPA Library quantification kit (Roche, ref. KK4824).
Equimolar pools of 9 libraries were constituted and sequencing was performed on an HiSeq2500 using the single read protocol (50nt) on 4 lanes.

Bioinformatics analysis

The quality of the data was assessed using FastQC from the Babraham Institute. Potential contaminants were investigated with the FastQ Screen software [22] from the Babraham Institute. RNA-seq 50nt reads were aligned to the *Aedes aegypti* assembly (AaegL5.0) with a set of gene model annotations (GCF_002204515.2_AaegL5.0_genomic.gff downloaded from NCBI on July 10 2018), using the splice junction mapper TopHat v2.1.1 [23], which used bowtie 2.2.8 [24]. Final read alignments having more than 3 mismatches were discarded. Gene counting was performed using HTSeq-count [25] version 0.9.0 (union mode). Since data come from a strand-specific assay, the read has to be mapped to the opposite strand of the gene. Before statistical analysis, genes with less than 15 reads (cumulating all the analysed samples) were filtered out. Dataset was then transformed in log CPM counts and a trimmed mean of M-values normalization was used to correct for libraries size effect. Description of the samples are present in the supplementary Files, File 1 under the name “Raw_Counts_RNASeq.txt" and File 2 under “sample.csv” respectively. A multidimensional scaling (MDS) was used to represent the the bray-curtis distances calculated between all replicates and samples on the 100 most expressed genes. Following steps were then followed, (i) Fit a quasi-likelihood negative binomial generalized log-linear model to count data. Conduct genewise statistical tests for a given coefficient or contrast (glmQLfit) (quasi-likelihood (QL) F-test against the FC threshold), (ii) Conduct genewise statistical tests for a given coefficient or contrast relative to a specified fold-change threshold (here a 2 fold change in expression threshold) (function glmTreat), (iii) Differentially expressed genes were identified using the Bioconductor [26] package edgeR 3.20.1 [27]. Data were normalized using the Relative Log Expression (RLE) [28] normalization factors. Genes with adjusted p-value less than 5% (according to the FDR method from Benjamini-Hochberg) were declared differentially expressed.

A list of candidate genes was obtained as the intersection between the lists of DE genes of each viral treatment. The CPM of each gene candidate was then plotted for each sample for visual inspection. Raw

count data and an R script generating all results and figures are available at the following address :https://github.com/loire/CCS_RNAseq_analysis/tree/master.

Results

Dataset exploration and quality control

To obtain a broad picture of the host response to DENV and RVFV infections, RNA-Seq was used to analyze differential gene expression at the mRNA level. The mRNA was isolated and purified from mock, RVFV and DENV infected cells at 1 and 6 days post infection to assess for early and late detection genes expression.

High-throughput sequencing generated an average of 11,021,430 million reads per sample. Approximately 94.63% of the reads were mapped to the *Aedes aegypti* genome (GCF_002204515.2_AaegL5.0_genomic.gff, [29]) and there were anywhere between 7028 and 9565 genes expressed per replicate at each time point. 9580 genes expressed above 0.5 count per millions in at least 3 samples were subsequently analyzed.

Selected samples exhibit a good homogenenity among samples after normalization. A nice dataset, with replicates well grouped and a net separation of groups of samples was observed (Figure 1).

The first dimension separates mock infection from viral infection, and the second dimension separates early (24 hours post infection) and late (6 days post infection) responses. Additionnaly, late responses to viral and mock infections are similar, indicating the possibility to conduct a direct comparison between them to search for common differential expression of genes in response to both viruses. For early response, both viruses have been analysed separately and search for overlap in list of differentially expressed genes was performed

Differential expression analysis

Early viral response

Expression values obtained at 24 h post infection in viral infected samples (DENV, RVFV) were compared to mock samples. Up-regulated genes detected in viral infections are, for the most part, related to native immune defense mechanisms. Family B and D clip-domain serine proteases (CLIP) are clearly overrepresented with 10 genes belonging to this family out of the 15 total genes (CLIP-B1, CLIP-B15, CLIP-B22, CLIP-B34, CLIP-B35, CLIP-B42, CLIP-B46, CLIP-D1, CLIP-D6, CLIP-E8). Prohibitin, a strongly conserved and ubiquitously expressed protein in eukaryotic cells, C-type lectins (CTLMA-13 and CTLMA-14), transferrin (Tf1), Peptidoglycan Recognition Protein (PGRP) and Gram negative binding protein (GNBP) have been also identified as up-regulated genes (Table 1).

Late viral response

Very interestingly, many of the up regulated genes detected in the early response are also upregulated 6 days post infection relative to control: five of the 10 Clip-domain serine proteases (CLIP-B15, CLIP-B34, CLIP-B35, CLIP-B46, CLIP-D1), transferrin (Tf1), C-type lectin (CTLMA-13 and CTLMA-14), PGRP and GNBP). On the top of this, 10 upregulated genes are common between the early and the late responses, Some genes have been found to be up-regulated only at the late phase of infection such as Niemann-Pick type C family genes, macroglobulin/complement and serine protease inhibitor (SRPN3) (Table 2).

Level of expression of up-regulated genes (early and late)

Genes up regulated in viral versus mock infection, at both late and early stages if infection, were looked for their intersection. Known genes that were filtered are presented Figure 2. Counts per million in each sample is used to check for actual overexpression in samples infected by a virus.

Late versus early response

Only two genes were found to be significantly upregulated between early and late response: F-spondin and nidogen. The latter is also related to viral infection [30] but is not upregulated when compared to mock infections in our data.

Discussion

Mosquitoes have been demonstrated worldwide to be vectors of several viral diseases of great importance for public health. Whole genome sequencing and annotation of the Zika, Chikungunya, RVF and Dengue vector, *Aedes aegypti*, has enabled a comparative phylogenomic analysis of the insect immune repertoire allowing deeper understanding of insect immune systems. Mosquitoes' innate immunity has evolved to recognize and respond to numerous pathogens, in a dynamic game where either host or pathogen is the winner [31]. Immune signaling pathways have been described both conservative and rapidly evolving features associated with different functional gene categories and particular aspects of immune reactions. Discovery of host factors regulated during viral infection of the mosquito may identify conserved protein families and pathways representing both mosquito anti-viral mechanisms as well as requirements for viral life cycles.

The main goal of our study was to identify up-regulated genes in *Aedes aegypti* in response to two major arboviruses infections, dengue and RVF infections by RNA sequencing.

Our comparative analysis highlighted the expression profile of 39 significantly upregulated genes following either early (27), late (22) or both early/late (10) indicating a potentially conserved transcriptomic signature of dengue and RVF emerging infections. Among annotated genes, Family B and family D clip-domain SPs (CLIP-B15, CLIP-B34, CLIP-B35, CLIP-B46, CLIP-D1), C-type lectins- mannose binding (CTLMA) namely CTLMA-13 and CTLMA-14 and transferrin are largely represented in the early/late viral up-regulated response we obtained. Peptidoglycan Recognition Protein (PGRP) and Gram negative binding protein (GNBP) have been also identified as up-regulated genes following both viral

infections at both early and late stages. Data supporting NTU discovery emphasizes the need for continuous amendments of the reference genome annotation [32]. Chymotrypsin-related SPs form a large family of enzymes that hydrolyze peptide bonds at different rates and with various degrees of specificity [33,34]. SPs and their serine protease homologs (SPHs) have been previously described to participate in digestion, defense, development, and other physiological processes. Like human clotting factors, they form complex networks to stop bleeding and fight infection. In each insect species with a known genome, SP-related proteins form a large family with 60–400 members [35-37]. In mosquitoes, numbers of clip-domain SPs/SPHs genes named CLIPs identified in genomes are 63 in *Aedes aegypti*, 55 in *Anopheles gambiae* and 45 in *Drosophila melanogaster* [31, 38]. They have been investigated for possible roles in antiparasitic responses and are known to regulate several invertebrate defense responses, including hemolymph coagulation, antimicrobial peptide synthesis, and melanization of pathogen surfaces [35-40]. Transferrin is also well known to be involved in viral responses [41, 42]. The C-type lectins (CTLs), have been implicated in immunity as opsonins and modulators of melanization. Prohibitin up-regulated at the early stage of infection is a strongly conserved and ubiquitously expressed protein in eukaryotic cells [43]. Niemann-Pick type C family and macroglobulin/complement genes have been already shown as related to dengue infections [44, 45].

Our results provide an extensive amount of data highlighting viral gene targets in the mosquito during infection. These preliminary findings have to be confirmed on experimentally RVF and DENV infected adult mosquitoes. Experiments involving a DENV and RVFV mouse model to show that virally up-regulated genes have an impact on the viral replicative cycles could also be performed.

The development of molecular diagnostic tools based on the detection of a combination of the different genes found to be up-regulated (CLIP-domain SP families, transferrin, prohibitin, C-type lectins) through innovative microarray/microbeads approaches is the next step.

Conclusions

This study is the first one, to our knowledge, to compare two major arboviral infections which are RVF and dengue infections that may occur in the same area and at the same time, as it is the case now in the Comoros archipelago, specifically on the island of Mayotte. Understanding the effects of infection on the mosquito, both common and unique to individual DENV and RVF, will aid in developing broadly applicable diagnostic tool to anticipate the occurrence of outbreaks.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article is available in a github repository (**https://github.com/loire/CCS_RNAseq_analysis**), along with scripts and instructions for a complete reproducibility of the analysis.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SL and CCS contributed to experimental design and supervised the study. MG, ED and EL contributed to the bioinformatic analysis. SL, CCS and EL wrote the manuscript. EC, SL, EL and CCS contributed to manuscript edition. All authors read and approved the final version of the manuscript.

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Tables

Table 1 : Early response up-regulated genes detected in viral infections. FC stands for Fold change; FDR for false discovery rate corrected pvalue.

NCBI gene ID	Gene name	logFC	FDR	Gene description
5563663	CLIPB35	3.06	1.12E-12	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5564201	CLIPB15	4.14	5.37E-11	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5564288	CTLMA14	4.01	4.20E-09	C-Type Lectin (CTL) - mannose binding. [Source:VB Community Annotation]
5578692		2.58	3.67E-07	Clip-domain serine protease [Source:UniProtKB/TrEMBL;Acc:Q1HQI3]
5570115		7.1	5.13E-06	trypsin-eta, putative [Source:VB Community Annotation]
5563616	CLIPB34	5.52	5.21E-06	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5574170		5.4	1.02E-05	serine protease [Source:VB Community Annotation]
5575350		6.45	3.50E-05	serine protease [Source:VB Community Annotation]
5565977	CLIPB46	3.61	4.70E-05	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5572333		6.15	6.90E-05	clip-domain serine protease, putative [Source:VB Community Annotation]
5575395		3.03	7.53E-04	prohibitin, putative [Source:VB Community Annotation]
5567561	CLIPB42	3.23	2.09E-03	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5575054		4.59	2.09E-03	serine protease, putative [Source:VB Community Annotation]
5575056	CTLMA13	6.06	2.32E-	C-Type Lectin (CTL) - mannose binding. [Source:VB

			03	Community Annotation]
5563566	CLIPB1	2.94	2.37E-03	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5578083		2.71	4.11E-03	F-spondin [Source:VB Community Annotation]
5576674		3.55	5.22E-03	ATP-binding cassette sub-family A member 3, putative [Source:VB Community Annotation]
5569658	CLIPD1	4.9	6.46E-03	Clip-Domain Serine Protease family D [Source:VB Community Annotation]
5570931	CLIPB22	4.09	7.35E-03	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5578380		2.24	7.47E-03	bm-40 precursor [Source:VB Community Annotation]
5567077	CLIPE8	4.87	7.91E-03	Clip-Domain Serine Protease family E. Protease homologue. [Source:VB Community Annotation]
5571998	PGRPS1	3.94	1.07E-02	Peptidoglycan Recognition Protein (Short) [Source:VB Community Annotation]
5573598	CLIPD6	3.27	1.44E-02	Clip-Domain Serine Protease family D [Source:VB Community Annotation]
5579417	Tf1	4.8	1.74E-02	transferrin [Source:VB Community Annotation]
5569420	GNBPA1	6.63	1.87E-02	Gram-Negative Binding Protein (GNBP) or Beta-1 3-Glucan Binding Protein (BGBP). [Source:VB Community Annotation]
5572428		4	3.56E-02	macroglobulin/complement [Source:VB Community Annotation]
5564141		3.21	4.92E-02	Niemann-Pick Type C-2, putative [Source:VB Community Annotation]

Table 2 : Late response up-regulated genes detected in viral infections. FC stands for Fold change; FDR for false discovery rate corrected pvalue.

geneID	Gene name	logFC	FDR	Gene description
110676293	LYSC11	2.91	1.35E-10	C-Type Lysozyme (Lys-A). [Source:VB Community Annotation]
5563663	CLIPB35	2.89	2.50E-10	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5564288	CTLMA14	4.07	9.29E-09	C-Type Lectin (CTL) - mannose binding. [Source:VB Community Annotation]
5565977	CLIPB46	5.05	1.50E-08	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5564201	CLIPB15	3.09	3.16E-06	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5574112		3.42	5.84E-05	GTP cyclohydrolase i [Source:VB Community Annotation]
5575350		6.38	6.41E-05	serine protease [Source:VB Community Annotation]
5574170		4.21	7.38E-05	serine protease [Source:VB Community Annotation]
5566832	SRPN3	7.02	1.66E-04	Serine Protease Inhibitor (serpin) likely cleavage at T/I. [Source:VB Community Annotation]
5578692		2.29	5.05E-04	Clip-domain serine protease [Source:UniProtKB/TrEMBL;Acc:Q1HQI3]
5572333		6.2	6.33E-04	clip-domain serine protease, putative [Source:VB Community Annotation]
5563725		4.67	1.50E-03	serine protease inhibitor, serpin [Source:VB Community Annotation]
5572968	RpS2	2.35	2.74E-03	40S ribosomal protein S2 [Source:UniProtKB/TrEMBL;Acc:Q1HRV1]
5569658	CLIPD1	4.81	6.39E-	Clip-Domain Serine Protease family D [Source:VB

			03	Community Annotation]
5574952		4.8	6.58E-03	metalloproteinase, putative [Source:VB Community Annotation]
5576150		3.55	7.79E-03	lipase 1 precursor [Source:VB Community Annotation]
5572428		5.11	7.79E-03	macroglobulin/complement [Source:VB Community Annotation]
5563616	CLIPB34	3.39	7.90E-03	Clip-Domain Serine Protease family B. [Source:VB Community Annotation]
5569420	GNBPA1	6.98	8.71E-03	Gram-Negative Binding Protein (GNBP) or Beta-1 3-Glucan Binding Protein (BGBP). [Source:VB Community Annotation]
5579417	Tf1	6.46	1.12E-02	transferrin [Source:VB Community Annotation]
5571998	PGRPS1	4.88	3.36E-02	Peptidoglycan Recognition Protein (Short) [Source:VB Community Annotation]
5575056	CTLMA13	4.31	4.11E-02	C-Type Lectin (CTL) - mannose binding. [Source:VB Community Annotation]

Figures

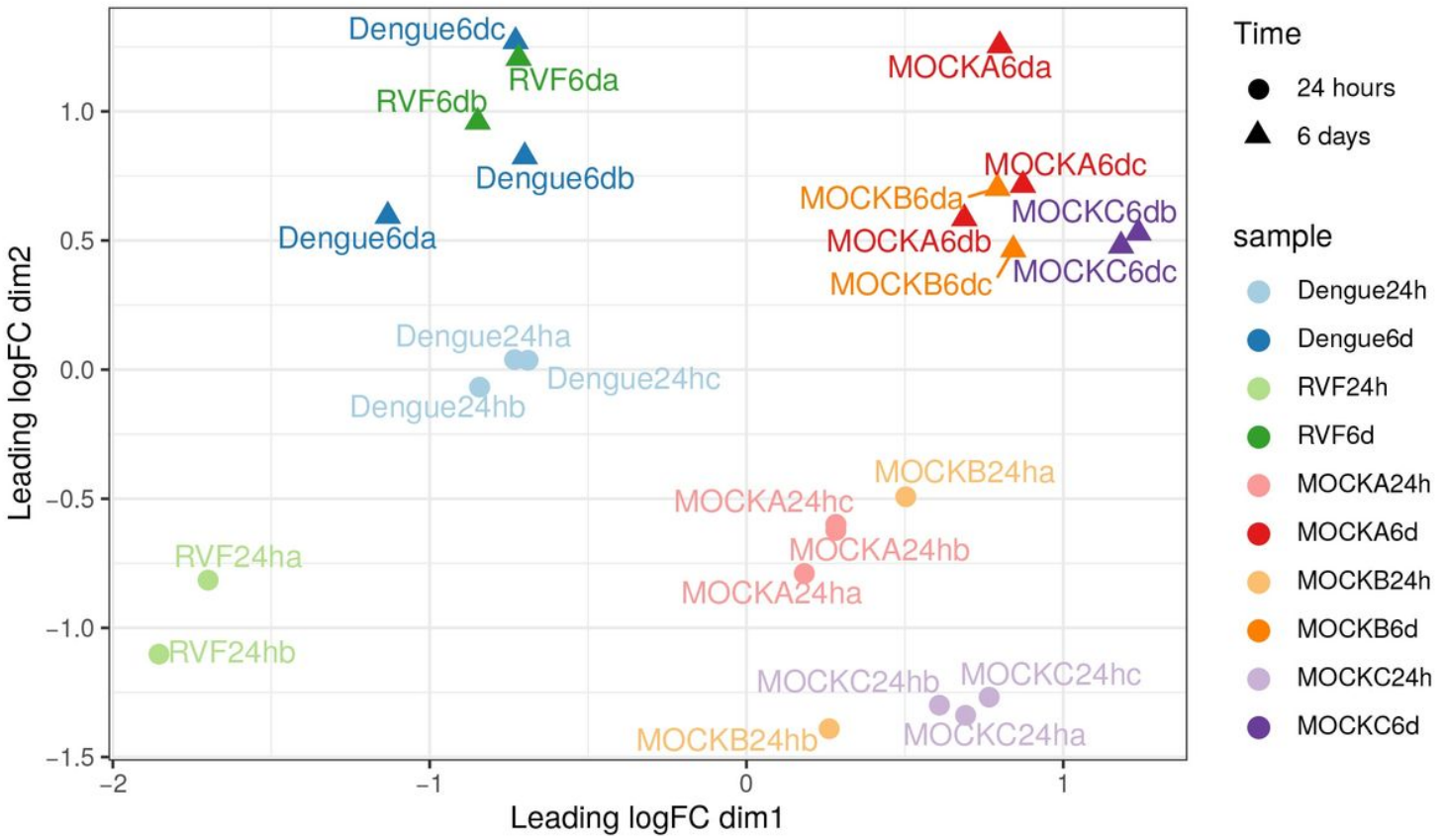

Figure 1

Non parametric multidimensionnal scaling (MDS) plot of filtered samples. Each point represent a sample. Color represent the type of sample (RVF : Rift valley fever). Mocks: negative controls of infections (see main text for description). Time post infection is represented with shapes of different color intensity : clear circle for early responses (24 hours); opaque triangles for late responses (6 days or 144 hours). Points clustered in space share a similar expression pattern on a subset of 100 highly expressed genes. Fold changes in gene expression are projected on two dimensions (axes). The first axe separates viral and mock samples, the second axe separates early (24 hours) and late (6 days) responses.

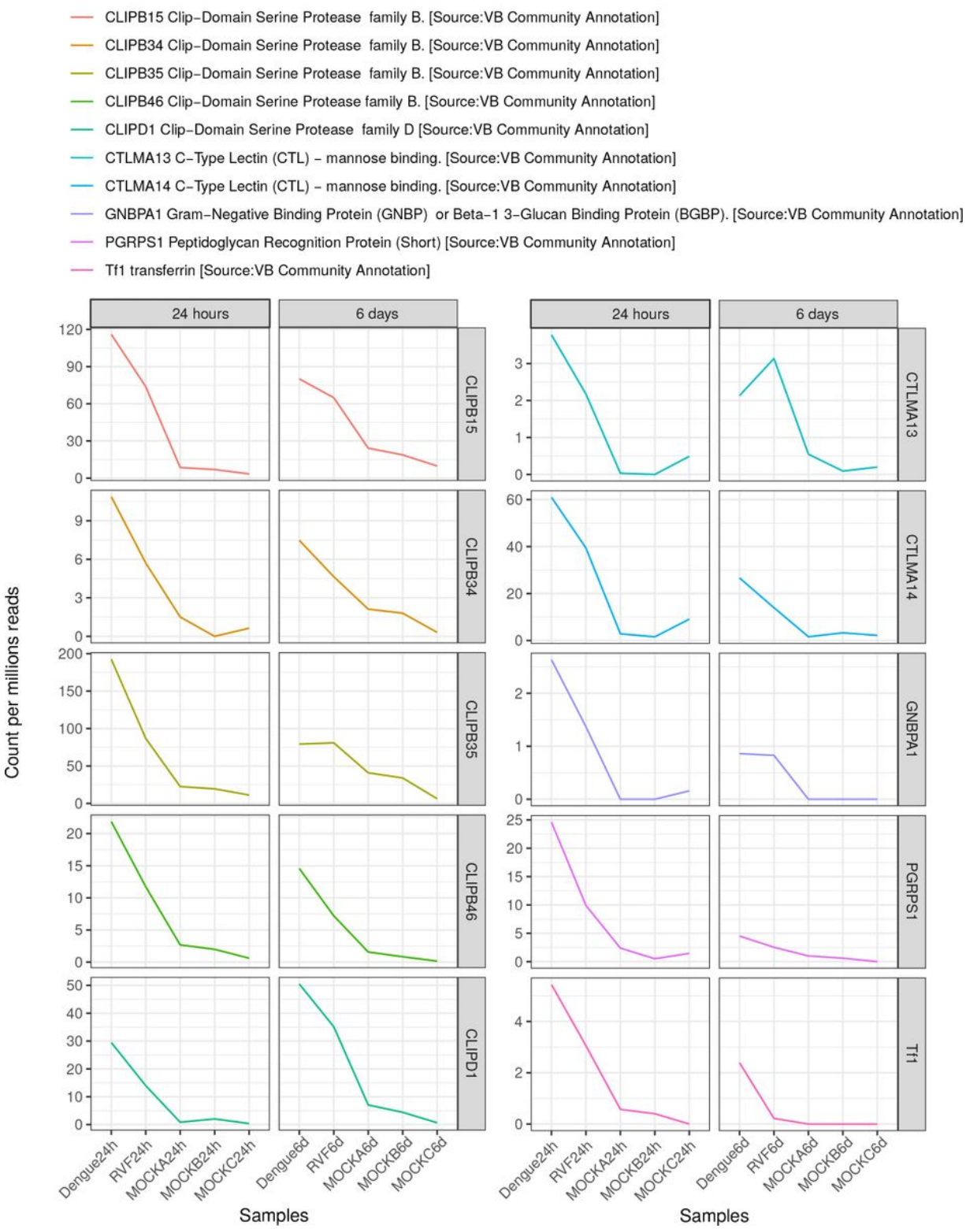

Figure 2

Counts per million in each sample is used to check for actual overexpression in samples infected by a virus. Each plot represent the mean expression level of a genes (expressed as count per million reads in the samples) among the replicates