RES-054

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Uncovering Dengue Virus Host Factors: Paving the Way for Innovative Antiviral Strategies

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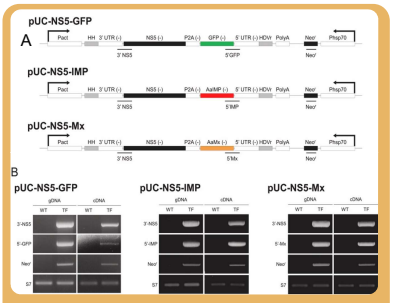


Fig. 1. Construction of the virus-inducible gene expression system
(A) The system contains negative-stranded *GFP* (pUC-NS5-GFP), *AaIMP* (pUC-NS5-IMP), and *AaMx* (pUC-NS5-Mx) and is regulated by *Drosophila* actin 5C promoter (Pact) and a polyA signal. The coding sequence of the negative-stranded self-cleaving P2A peptide was inserted to separate the NS5 and target proteins. Hammerhead (*HH*) ribozyme and hepatitis delta virus ribozyme (*HDVr*) ensured the generation of correct negative-stranded DENV2 3' and 5' UTRs. The neomycin resistance gene *Neo*^{r}, regulated by *Drosophila* heat shock protein 70 promoter (*Phsp70*), allowed for selection of stable cell lines.
(B) Diagnostic PCRs of *A. aegypti* (ATC10) stable cell lines containing the gene expression system. Both genomic DNA (gDNA) and total RNA were extracted from parental ATC10 (WT) cells and the stable cell lines (TF). 3'-NS5 corresponds to primer pairs that recognized the junction between 3' UTR and NS5. 5'-GFP, 5'-IMP, and 5'-Mx correspond to primer pairs that recognized the junction between 5' UTR and the target gene (*GFP*, *AaIMP*, and *AaMx*, respectively). WT ATC10 cells were used as the PCR control, and *A. aegypti* ribosomal protein S7 was used as the internal control.

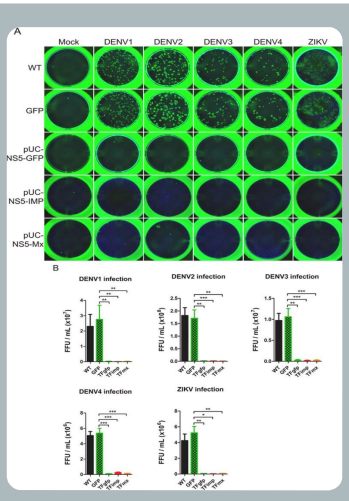


Fig. 4. Viral titer in pUC-NS5-GFP-, pUC-NS5-IMP-, and pUC-NS5-Mx-transfected ATC10 cells
(A) ATC10 cells transfected with pUC-NS5-GFP, pUC-NS5-IMP, or pUC-NS5-Mx and WT ATC10 cells were infected with different flaviviruses (DENV1–4 or ZIKV) at a multiplicity of infection (MOI) of 1. Four days post infection, the cell culture medium of each group was subjected to a focus-forming assay. Anti-flavivirus NS1 antibody (green) was used in this assay.
(B) Viral titer was quantified for each group from at least three biological repeats. Representative images are shown in (A). Data are means ± SDs. Comparisons were performed using the Mann–Whitney test; * $p \le 0.05$; ** $p \le 0.01$; *** $p \le 0.001$.

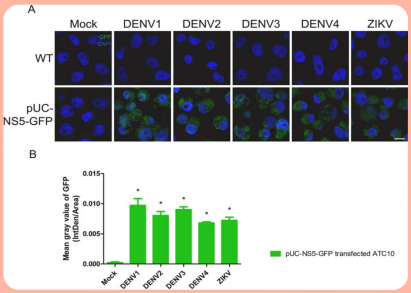


Fig. 2. Expression of GFP in the virus-inducible gene expression system
(A) pUC-NS5-GFP-transfected ATC10 cells were infected with different flaviviruses (DENV1–4 or ZIKV) at a multiplicity of infection (MOI) of 1. Four days post infection, the cells were fixed and the expression of GFP was determined via immunofluorescence assay. Anti-GFP antibody (green) and DAPI (blue) were used to localize the expression of GFP and the nucleus, respectively. Scale bar = 5 μm.
(B) Quantification of GFP protein level in pUC-NS5-GFP-transfected ATC10 cells after infection with the flaviviruses. Representative images are shown in (A). Data in (B) are means ± SDs. GFP protein level was quantified for each group from at least three biological repeats. Comparisons were performed using the Mann–Whitney test with a mock infection group; * $p \le 0.05$.

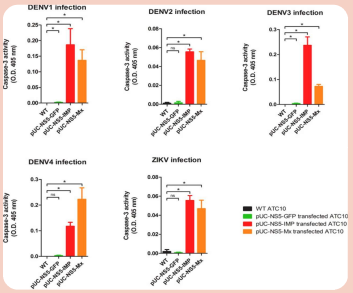


Fig. 3. pUC-NS5-IMP- and pUC-NS5-Mx-transfected ATC10 cells activated effector caspase activity after flavivirus infection
ATC10 cell lines transfected with pUC-NS5-GFP, pUC-NS5-IMP, or pUC-NS5-Mx and WT ATC10 cells were infected with different flaviviruses (DENV1–4 or ZIKV) at a MOI of 1. Three days post infection, cell lysates were collected and resuspended in cell lysis buffer. Equal quantities of total protein were subjected to caspase activity assays. Mock signal was used as the basal signal and subtracted from each flavivirus infection group. Effector caspase activity was quantified for each group from at least three biological repeats. Data are means ± SDs. Comparisons were performed using the Mann–Whitney test; ns, non-significant; * $p \le 0.05$.

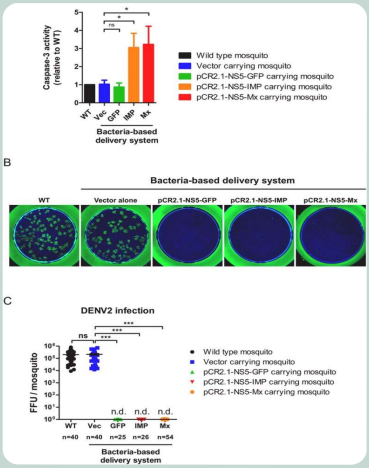
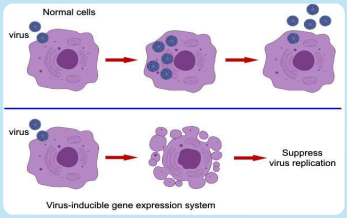


Fig. 5. The virus-inducible system promotes antiviral activity in mosquitoes
(A) WT mosquitoes, mosquitoes carrying empty vector, and mosquitoes carrying the virus-inducible system were infected with DENV2 via thoracic injection (2×10^3 FFU/mosquito). Four days after infection, total mosquito protein was collected. Equal quantities of total protein were subjected to caspase-3 activity assays. Mock signal was used as the basal signal and subtracted from each group. Quantitative measurements were conducted in triplicate and normalized against the WT signal. Data are means ± SDs. Comparisons were performed using the Mann–Whitney test; ns, non-significant; * $p \le 0.05$.
(B) WT mosquitoes, mosquitoes carrying empty vector, and mosquitoes carrying the virus-inducible system were infected with DENV2 via thoracic injection (2×10^3 FFU/mosquito). Four days after infection, the mosquitoes were collected and subjected to focus-forming assays. Anti-flavivirus NS1 antibody (green) was used in this assay. Representative images are shown.
(C) Viral titer was quantified for each group from at least three biological repeats. Data are means ± SDs. Comparisons were performed using Dunn's multiple comparison test; *** $p \le 0.001$.



Conclusion

- A virus-inducible gene expression system was developed to block virus replication and transmission.
- The system was activated upon dengue and Zika viruses infection, leading to the production of two apoptosis-related genes.
- A bacteria-based delivery approach was established to generate mosquitoes with antiviral activity.