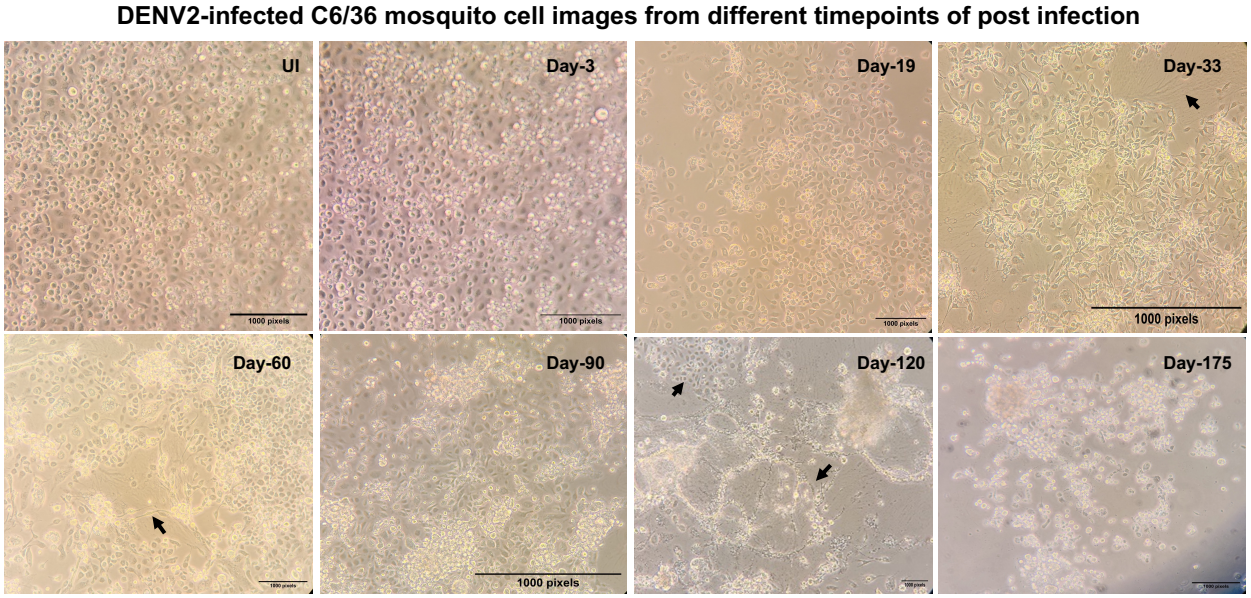
SUPPLEMENTAL INFORMATION

Persistent and Long-Term Infectivity of Dengue Virus in Mosquito Cells Revealed Reduced Replication in Vector Host and Human Endothelial Cells

Swarnendu Basak, Md Bayzid, Girish Neelakanta, Hameeda Sultana *

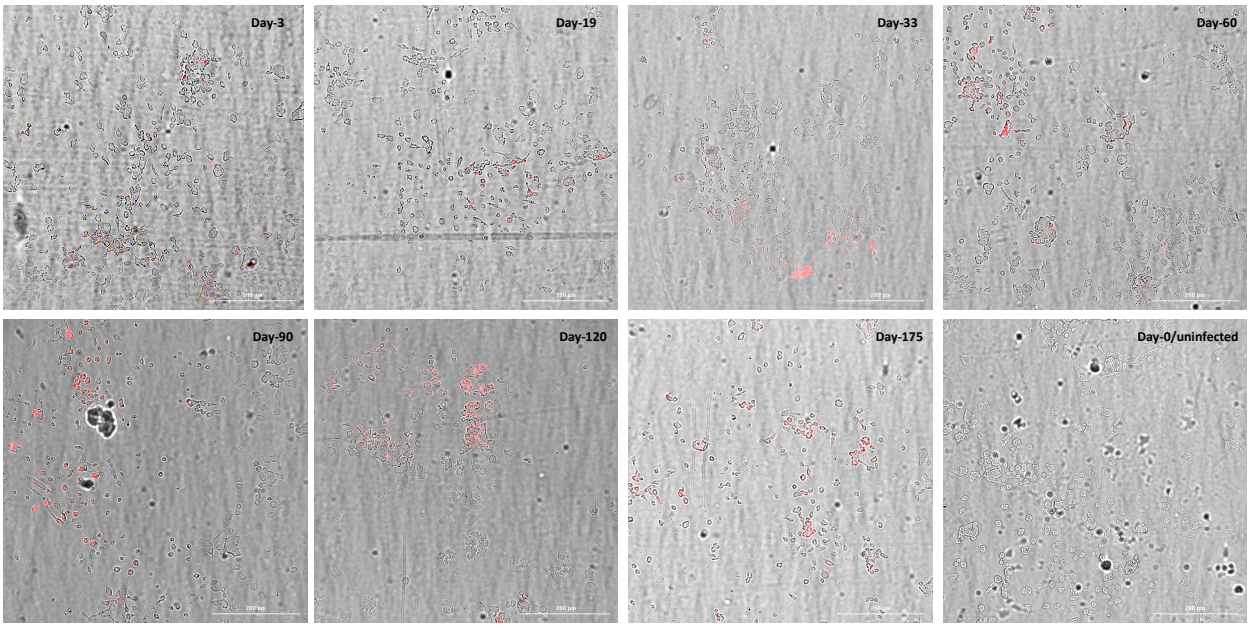
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SUPPLEMENTAL FIGURES AND LEGENDS

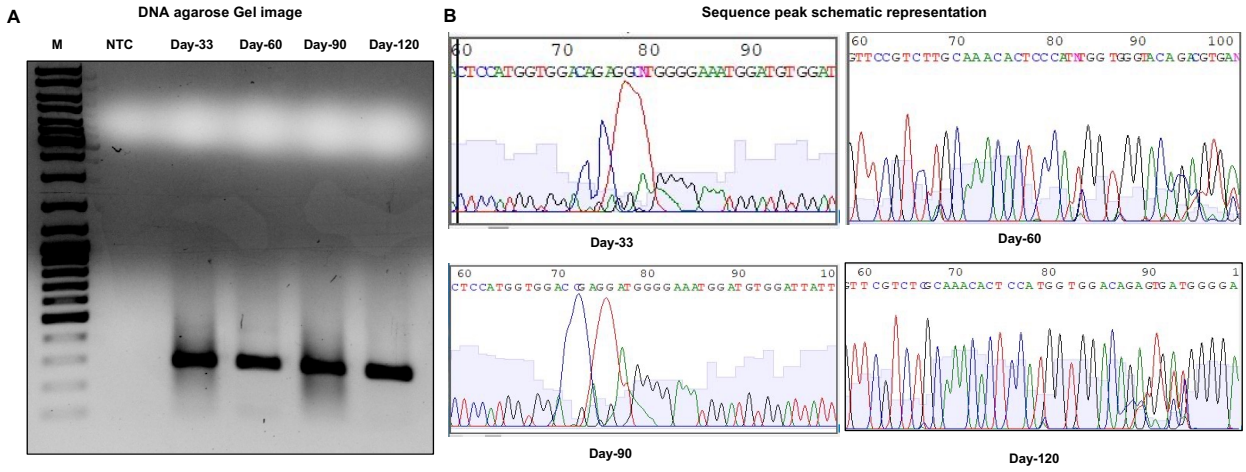


Supplemental Figure S1: DENV2 persistent infection and morphology of mosquito cells on different days. Bright field images from C6/36 cells were collected as uninfected (before infection) or after DENV2 infection on days of 3, 19, 33, 60, 90, 120 and 175 post infection. All images were obtained before the collection of supernatants as viral supernatants on these different days. The bright field image data was obtained using Samsung Galaxy S21 Ultra smartphone camera. Scale calibration was performed in ImageJ.

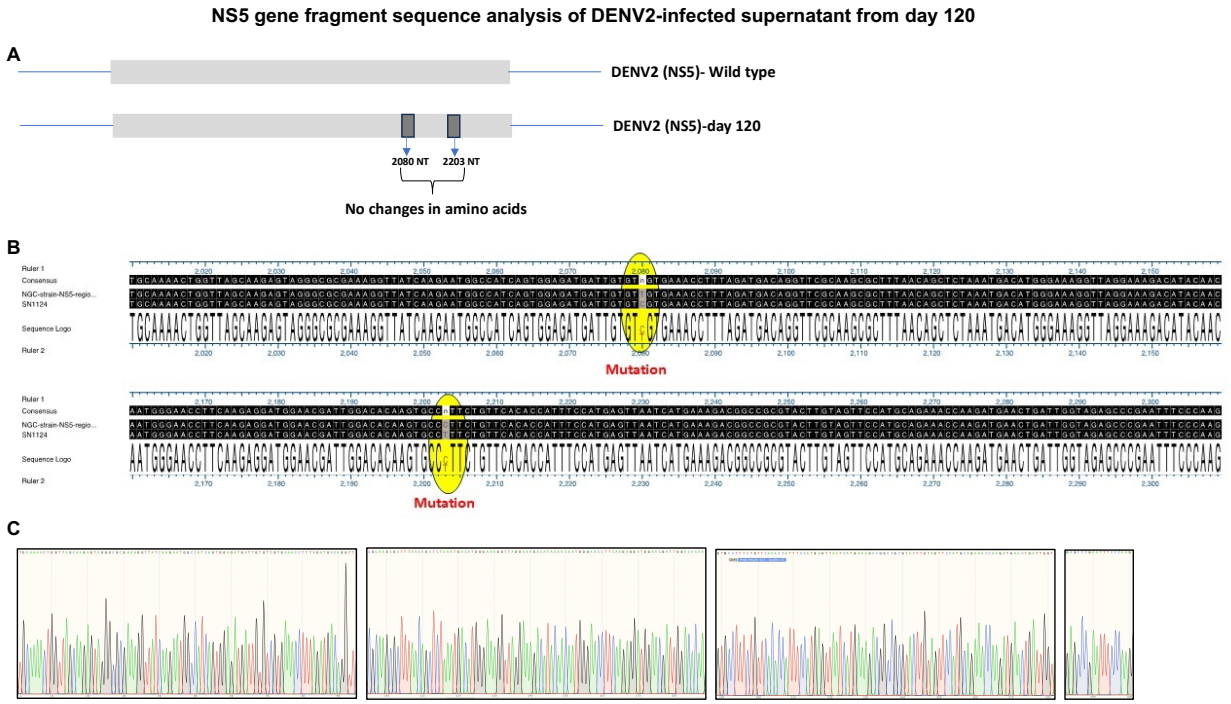
High resolution of merge images from viral infectivity assay from days 3 to 175 post DENV2-infection in C6/36 mosquito cells



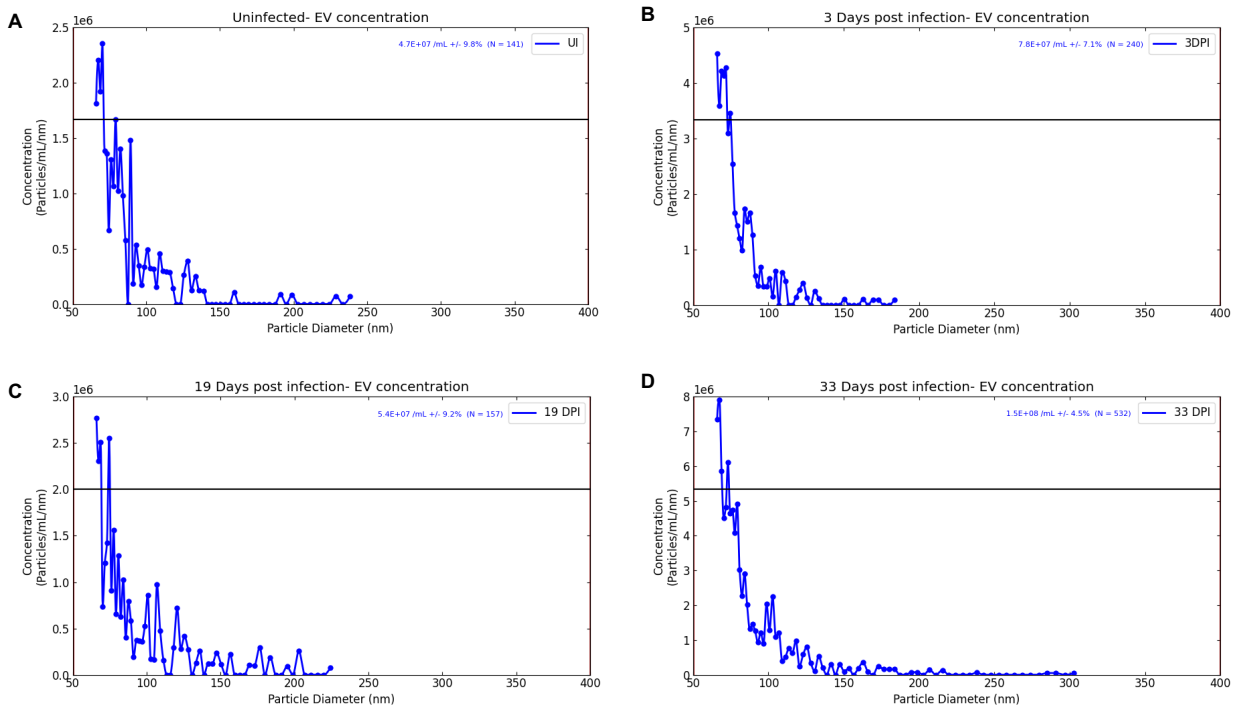
Supplemental Figure S2: Enlarged merged images from viral infectivity assay performed on viral supernatants. Bright field and red fluorescence images from C6/36 cells collected as uninfected (before infection) or after DENV2 infection for 5 days with viral supernatants from 3, 19, 33, 60, 90, 120 and 175 are shown.



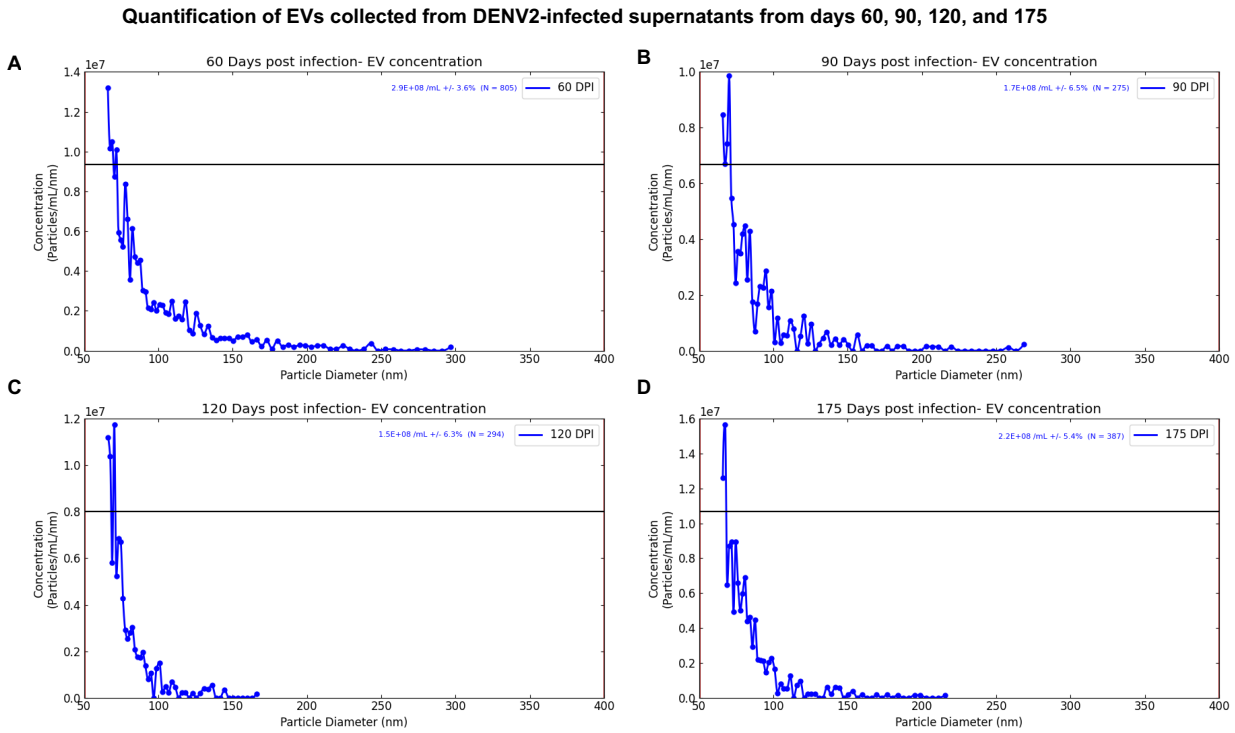
Supplemental Figure S3: Sequence analysis of Envelope (E) gene fusion loop from DENV2-infected viral supernatants. DNA agarose gel image showing the amplified fusion loop product from the four viral supernatants (A). Entire fusion loop region from viral supernatants obtained from days 33, 60, 90 and 120 was sequenced and the chromatogram peaks (B) are shown along with the respective sequences. Numbers represents 10 nucleotides in each sequence. M indicates marker and NTC represents no template control.



Supplemental Figure S4: Sequence analysis of Non-Structural 5 (NS5) gene from DENV2-infected viral supernatants. Entire NS5 gene schematic representation for wild-type or day 120 viral supernatant is shown (A). A fragment of NS5 gene sequence analysis is shown with two nucleotide substitutions in day 120 viral supernatant (B). Sequence is shown with nucleotides changes leading to non-sense mutations with no amino acid substitutions is represented in yellow circle (B). Chromatogram peaks are shown along with the respective sequences (C).



Supplemental Figure S5: Concentration and sizes of EV-derived from viral supernatants collected at early timepoints. Concentration and sizes of EV-derived from viral supernatants from uninfected, or days 3, 19 and 33 are shown (A-D). The nCS1 analyzer generated graphical representations showing EV concentrations and diameter of each particle. One sample in duplicate was considered for measuring EV numbers and concentration.



Supplemental Figure S6: Concentration and sizes of EV-derived from viral supernatants collected at later timepoints. Concentration and sizes of EV-derived from viral supernatants from uninfected, or from days 60, 90, 120 and 175 are shown (A-D). The nCS1 analyzer generated graphical representations showing EV concentrations and diameter of each particle. One sample in duplicate was considered for measuring EVs numbers and concentration.