Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Smartphone Clip-On Instrument and Microfluidic Processor for Rapid Sample-to-Answer Detection of Zika Virus in Whole Blood Using Spatial RT-LAMP

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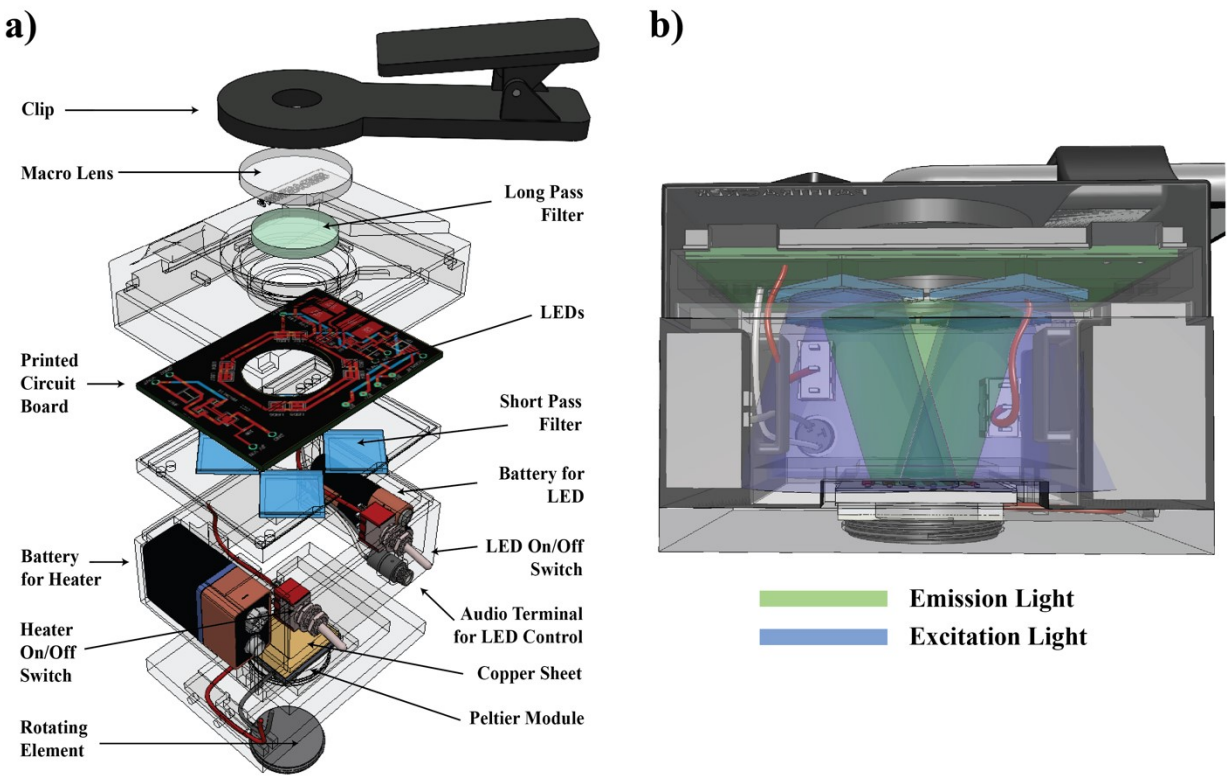

Fig. S1. Schematic of the instrument and the working principle. **a)** The bottom portion of the instrument is designed to house the heating element, Module B, and the rotating cap. The heating element is fabricated by attaching a copper sheet to the Peltier module. When screwed in, the rotating cap ensures the direct contact of the surfaces of the copper sheet and the Module B for uniform and stable heat transfer. The central portion of the instrument is designed to house batteries for powering the Peltier module and the LEDs. The uppermost portion of the instrument has an opening at the center where the fluorescence emission from the amplification reactions passes through a long pass filter and a macro lens on the path toward the smartphone camera. Within the upper compartment, there are four 490 nm short-pass filters, a printed circuit board for voltage control of the LEDs, and a filter holder to create and control the excitation illumination from eight LEDs with a center wavelength of 485 nm; **b)** Working principle of the instrument. The LED illumination is directed toward Module B to excite the EvaGreen dye during the amplification reactions. The 525 nm long-pass filter blocks the excitation light while allowing only the emission wavelength to pass through. The macro lens reduces the focusing distance between the smartphone camera and the surface of Module B.

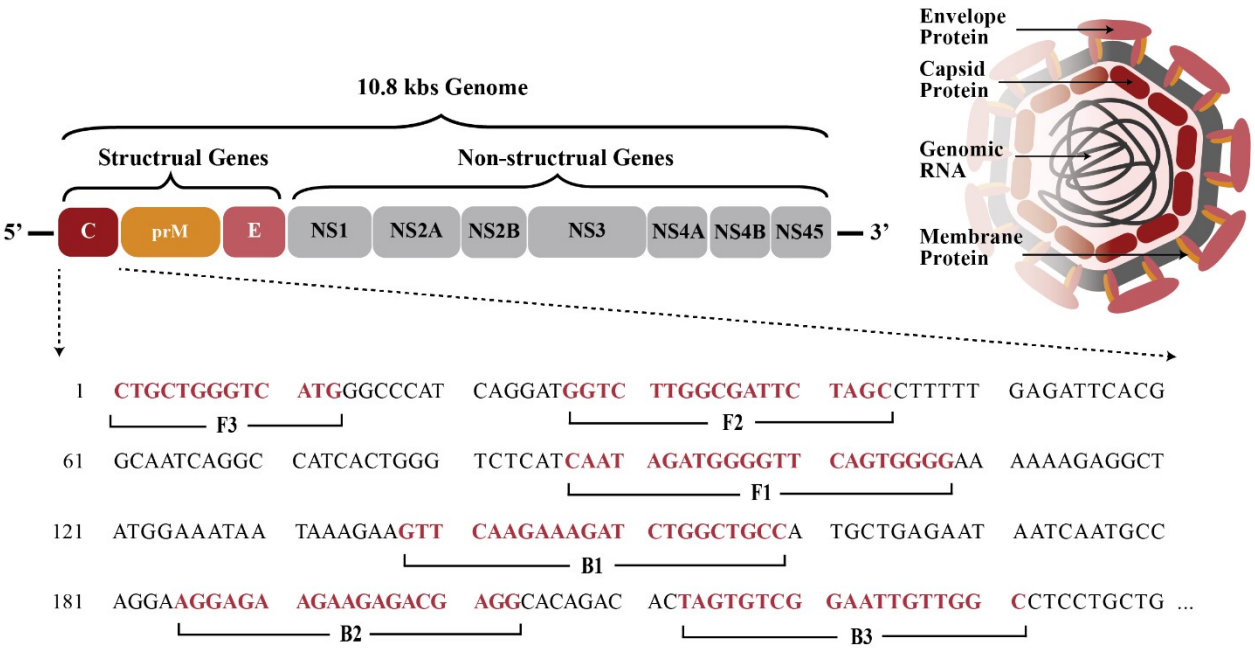

Fig. S2. The genome of the ZIKV is a 10.8 kbs long single-stranded RNA (ssRNA) that consists of a structural and non-structural parts of the genes. The structural gene encodes capsid proteins, precursor membrane proteins, and envelope proteins. Within the capsid protein, the selected primers target the six different regions of the RNA (F1, F2, F3, B1, B2, and B3).

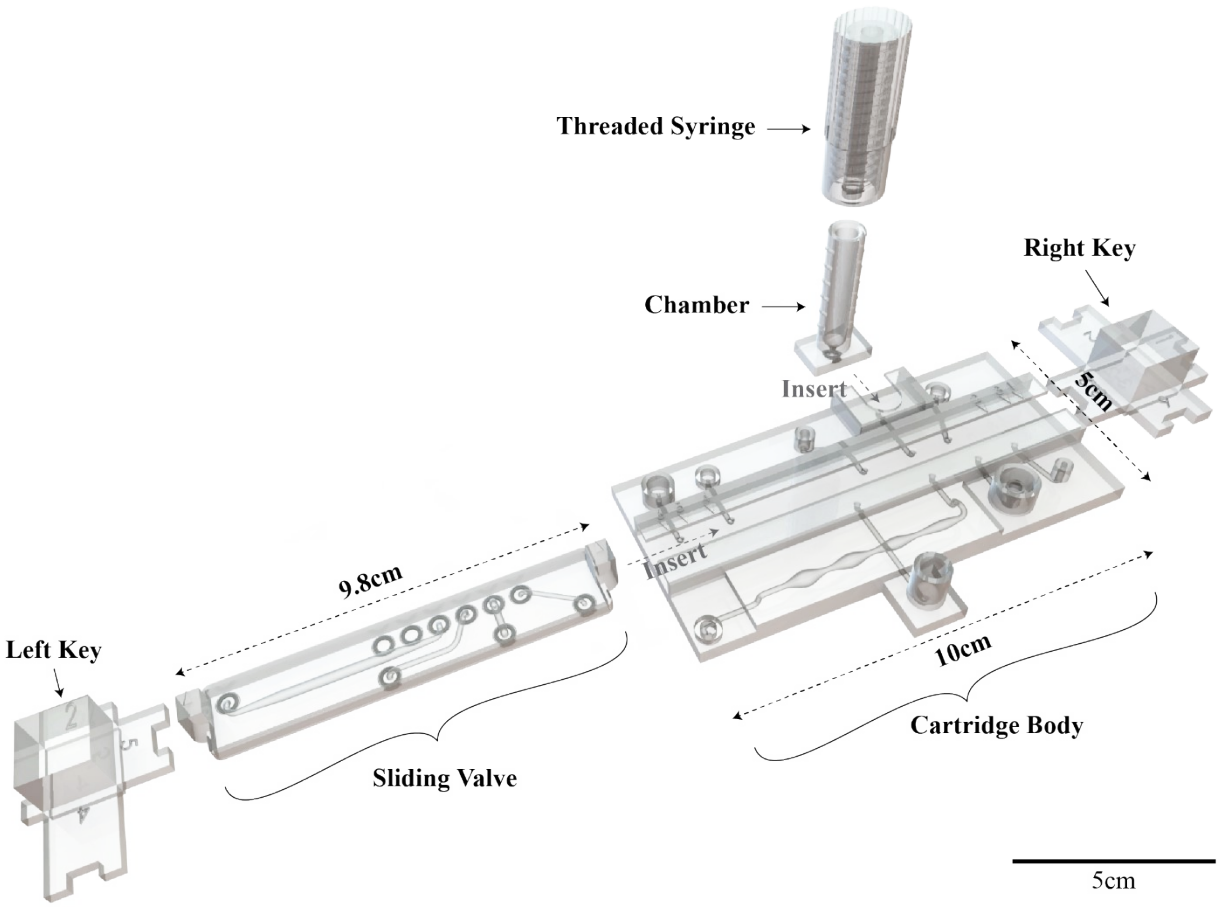

Fig. S3. Schematic of Module A, which includes the sliding valve, the cartridge body, and the threaded syringe with the chamber.

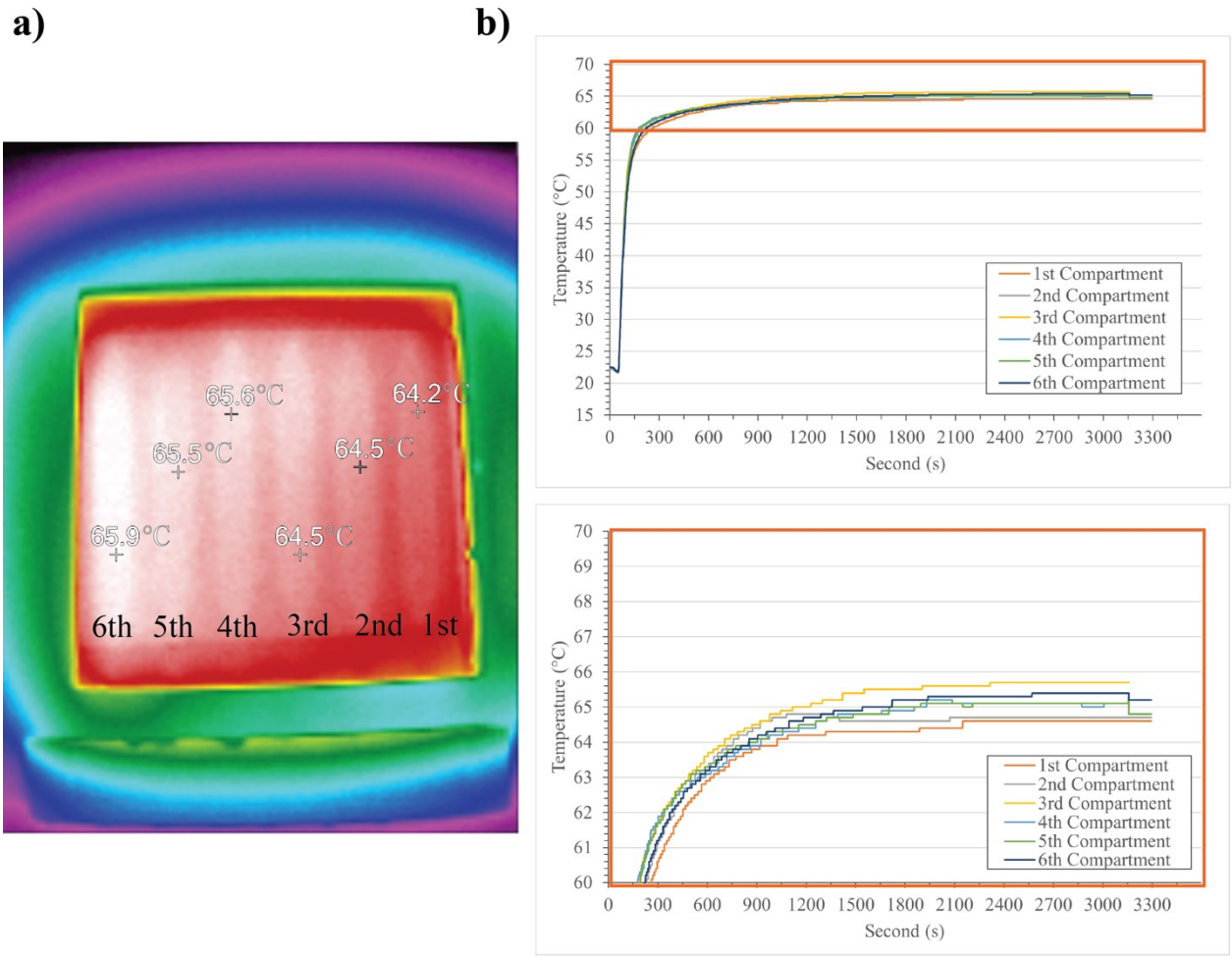

Fig. S4. Temperature characterization of Module B. **a)** Temperature measurement of each compartment of Module B using an infrared thermal imager; **b)** Monitoring of temperature of each compartment using thermocouple thermometer.

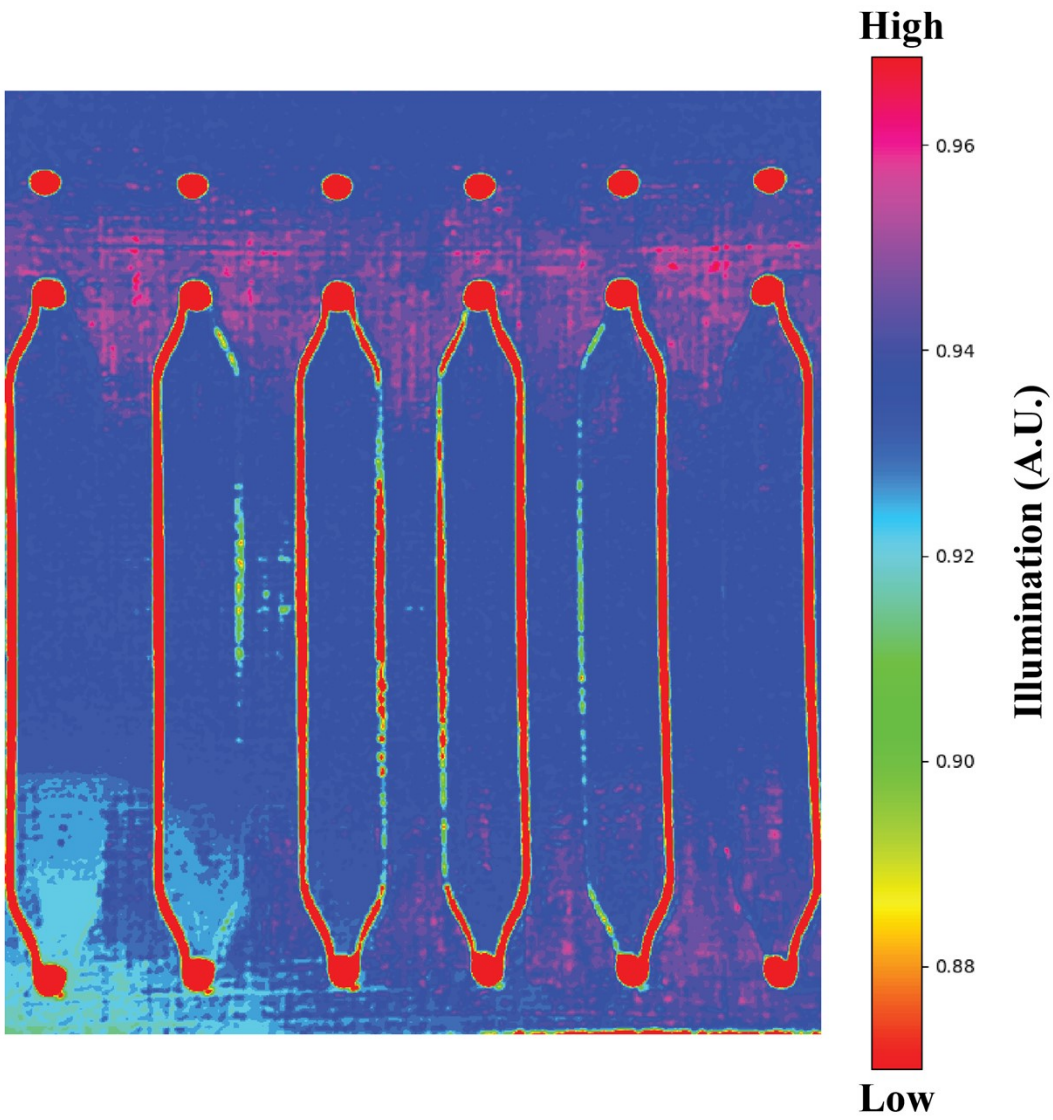

Fig. S5. Excitation light illumination histogram. The illumination intensity over the surface of Module B is characterized. The LEDs are uniformly illuminating the surface of Module B with a maximum intensity difference of approximately 0.05 a.u. between the brightest and darkest regions.

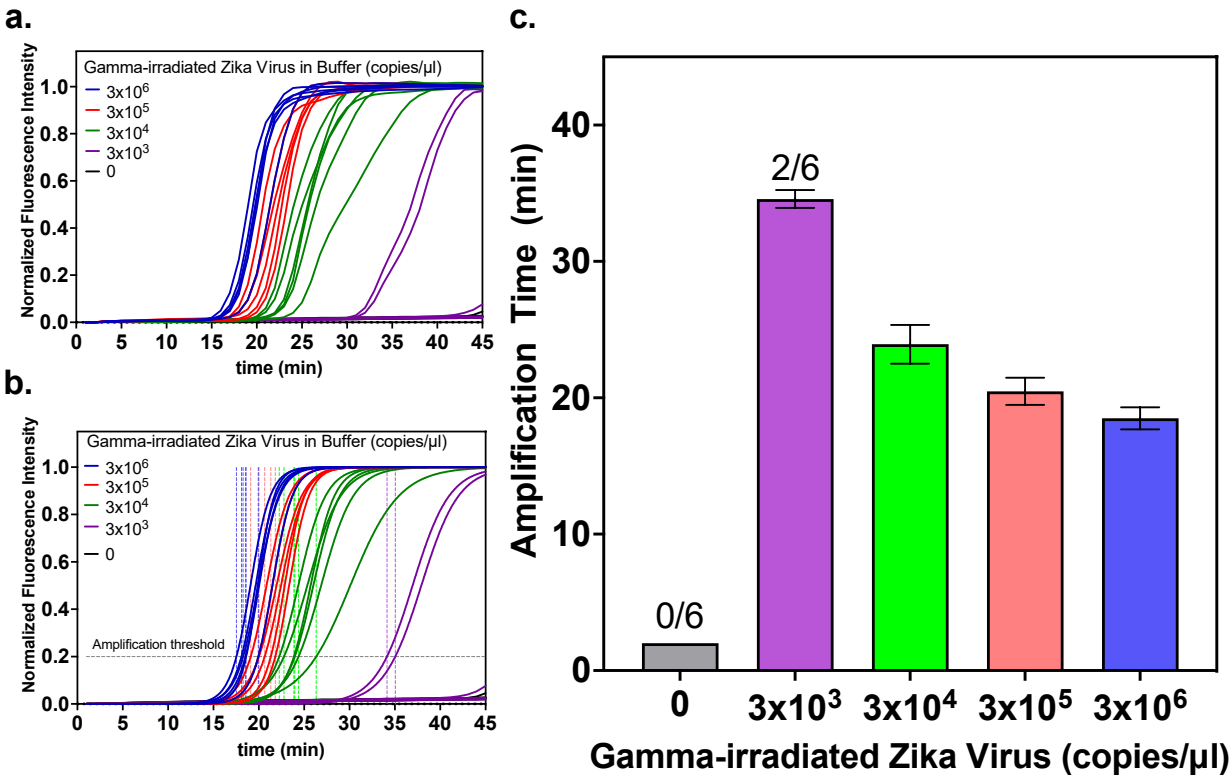

Fig. S6. Off-cartridge amplification results for gamma-irradiated Zika virus strain PRVABC59 in buffer. **a)** Normalized and **b)** fitted amplification curves; **(c)** Bar plot of amplification times. Number over top of columns signifies number out of total replicates amplified for concentrations where not all replicates amplified.

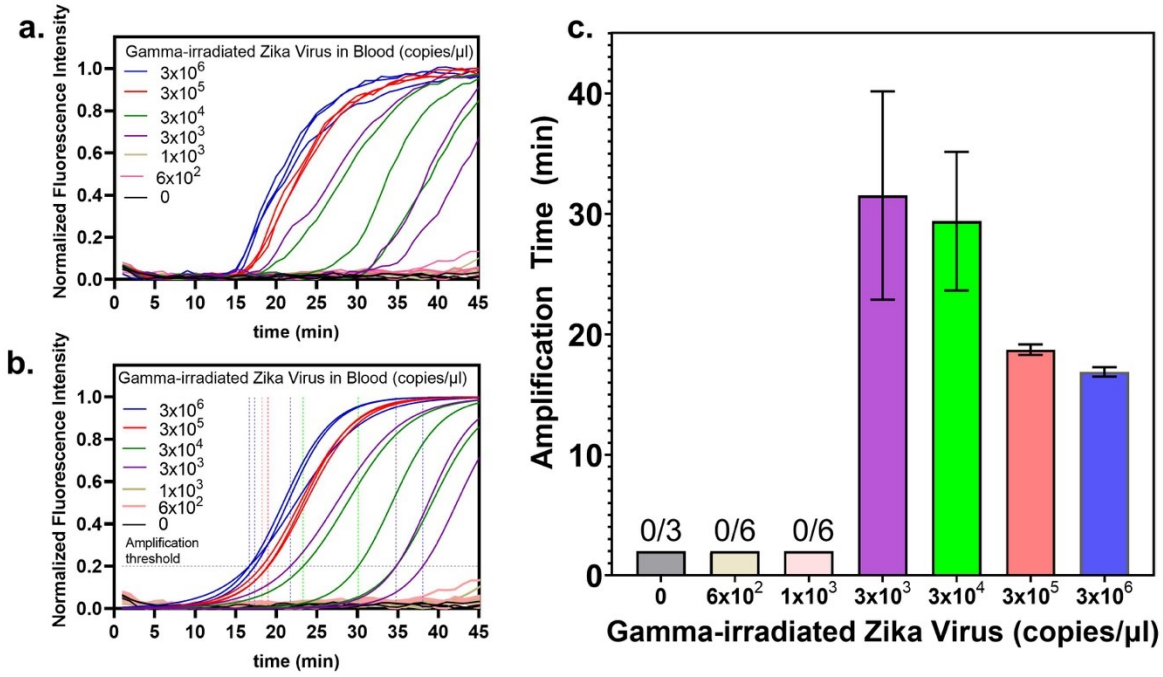

Fig. S7. Off-cartridge amplification results for gamma-irradiated Zika virus strain PRVABC59 in whole blood. **a)** Normalized and **b)** fitted amplification curves; **(c)** Bar plot of amplification times. Number over top of columns signifies number out of total replicates amplified for concentrations where not all replicates amplified.

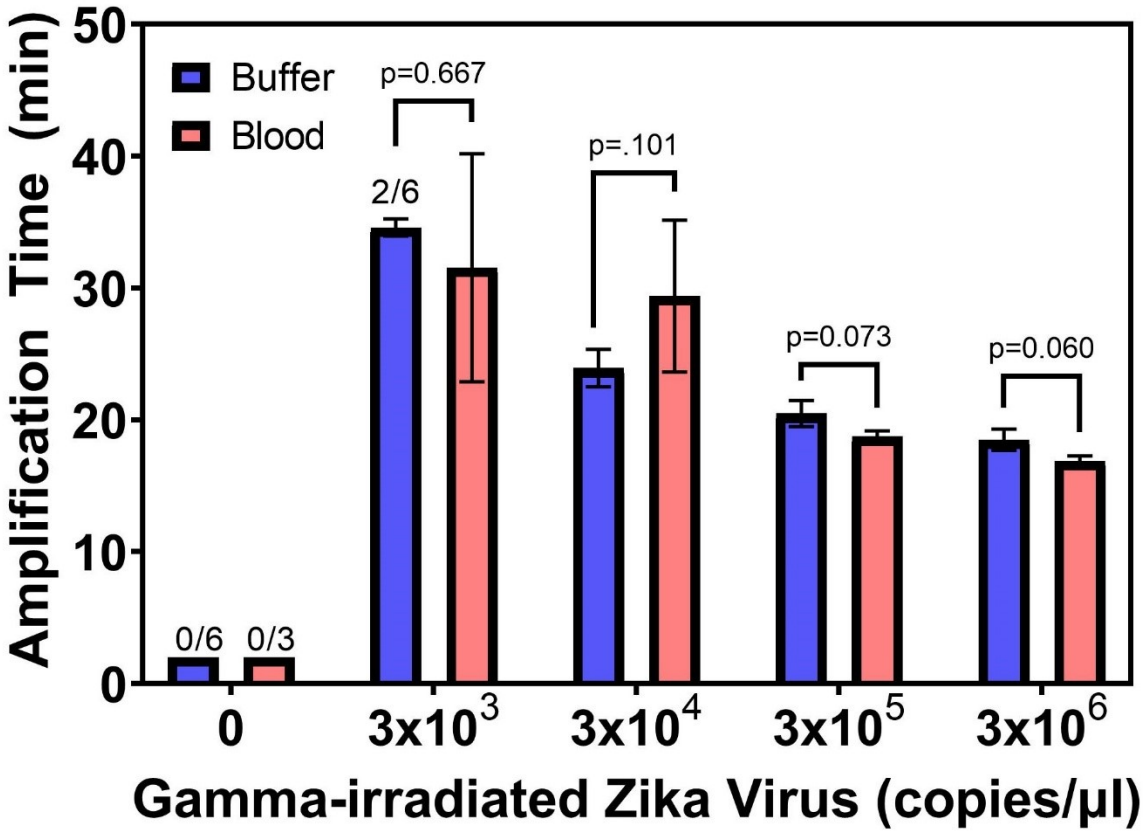

Fig. S8. Comparison of off-cartridge amplification times of gamma-irradiated Zika virus strain PRVABC59 in blood and buffer. Number over top of columns signifies number out of total replicates amplified for concentrations where not all replicates amplified.

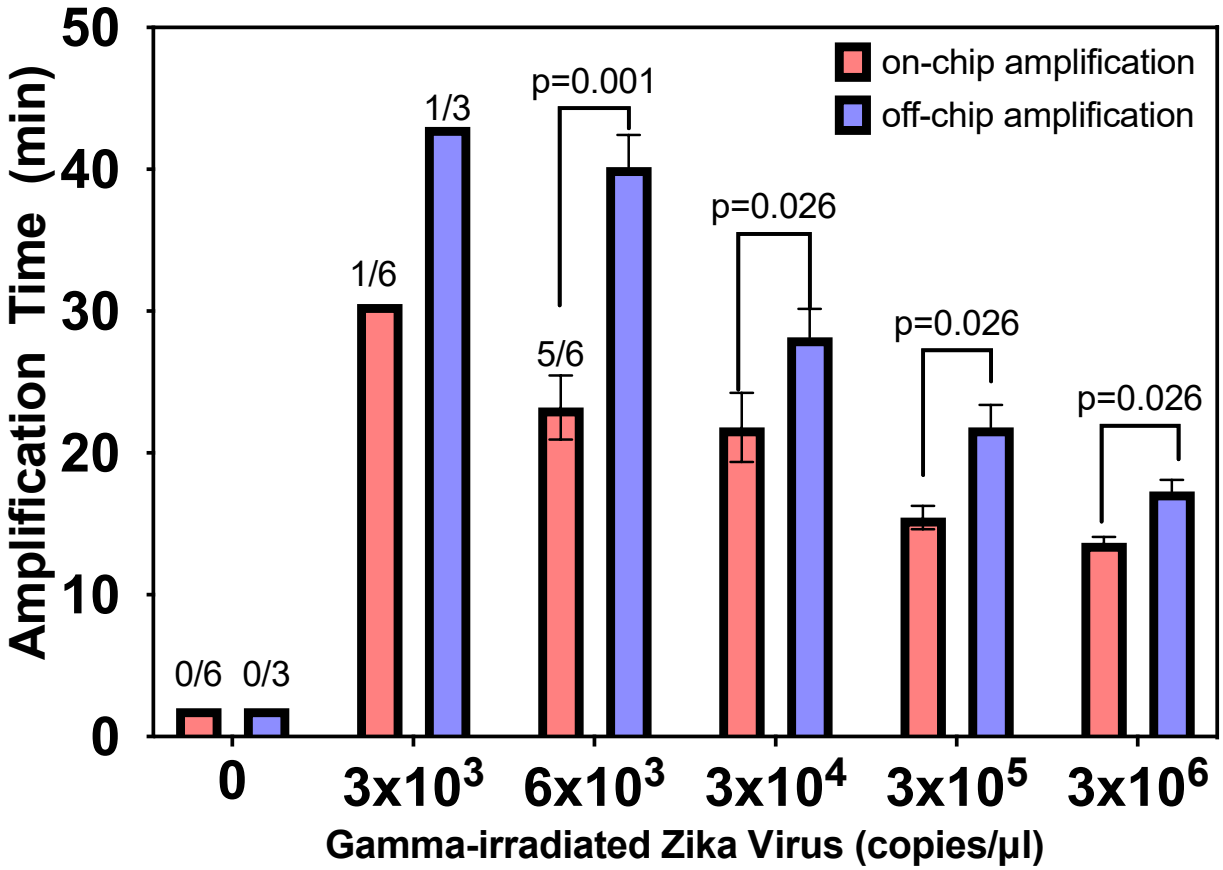

Fig. S9. Comparison of on-cartridge and off-cartridge amplification times of gamma-irradiated Zika virus strain PRVABC59 in blood. On-cartridge results used are from global intensity analysis. Number over top of columns signifies number out of total replicates amplified for concentrations where not all replicates amplified.

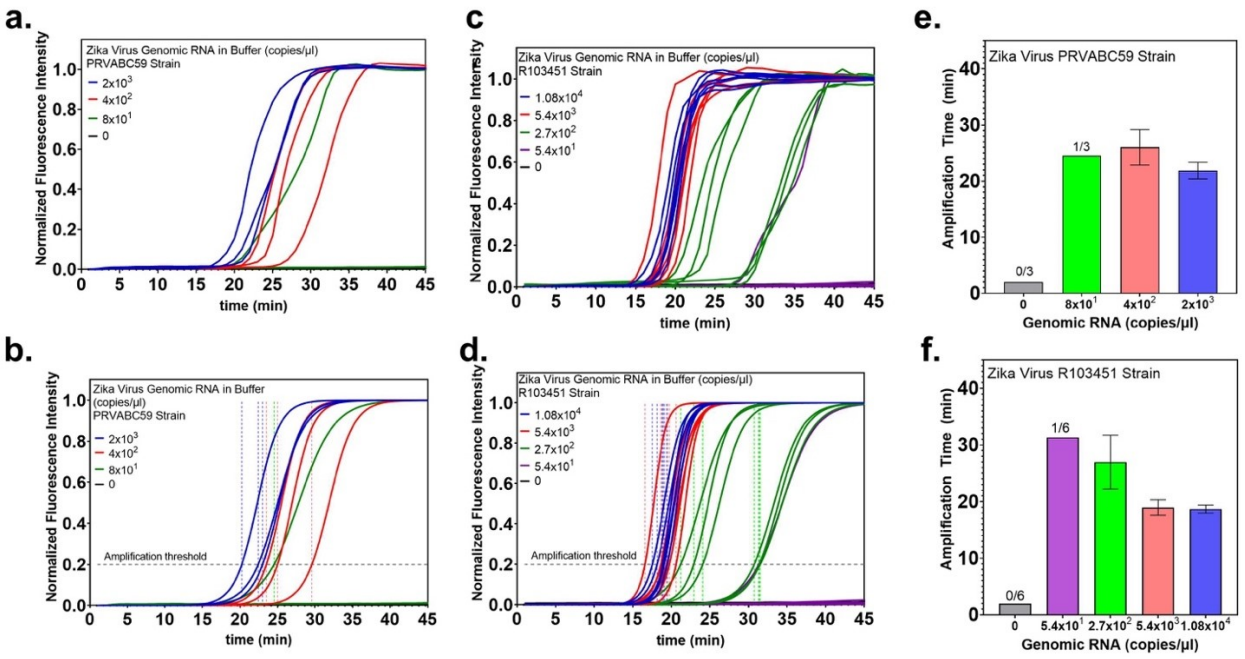

Fig. S10. Off-cartridge amplification results for genomic Zika RNA in buffer. **a)** Normalized and **b)** fitted amplification curves for Zika strain PRVABC59; **c)** Normalized and **d)** fitted amplification curves for Zika strain R103451; **e-f)** bar plot of amplification times for Zika virus strains **e)** PRVABC59 and **f)** R103451, respectively. Number over top of columns signifies number out of total replicates amplified for concentrations where not all replicates amplified.

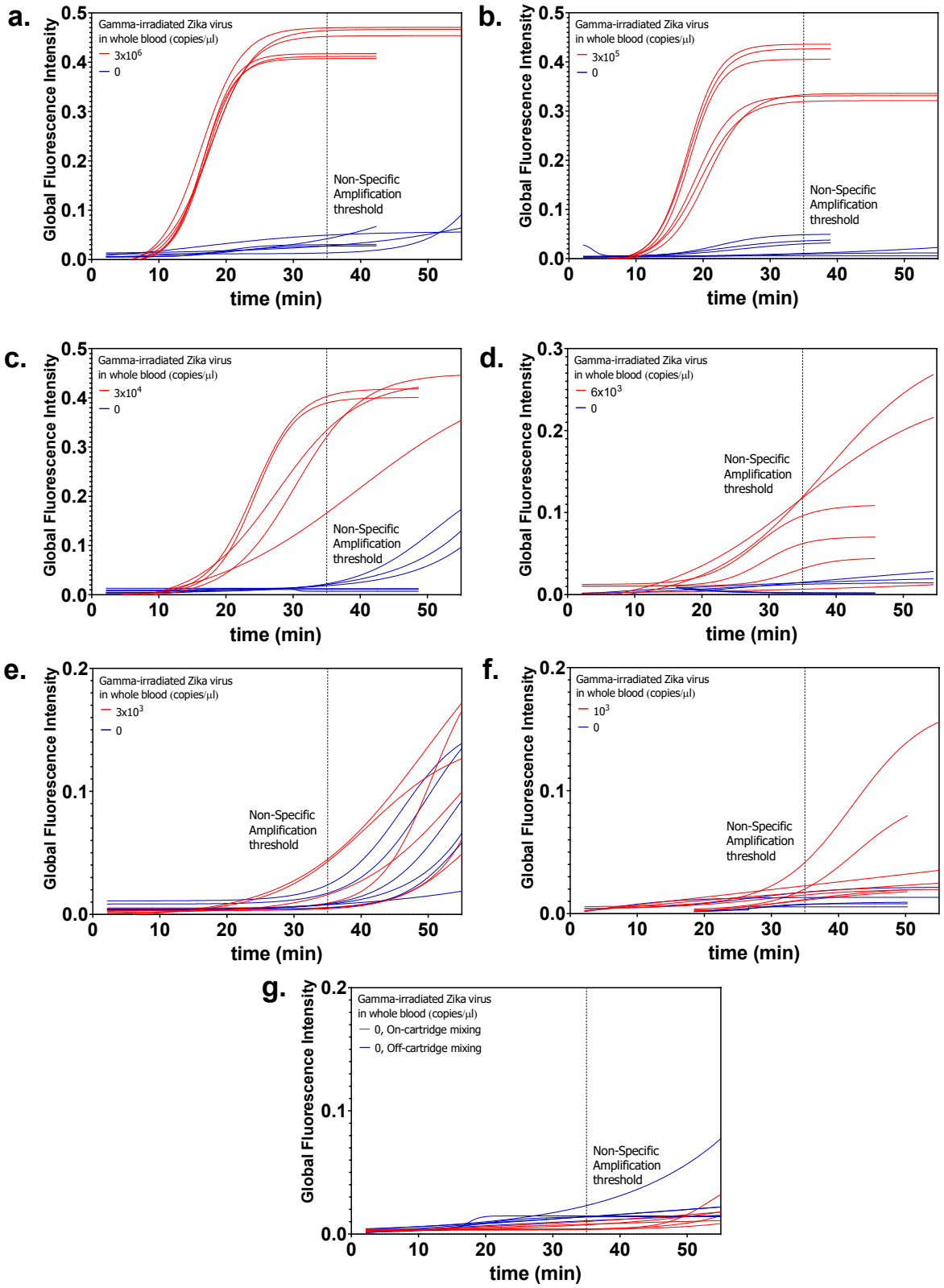

Fig. S11. On-cartridge curve-fitted data from *PathTracker* app for global intensity analysis. Gamma-irradiated Zika virus in whole blood: **a)** $3x10^6$; **b)** $3x10^5$; **c)** $3x10^4$; **d)** $6x10^3$; **e)** $3x10^3$; **f)** $1x10^3$; and **g)** 0 (negative control) copies/µl.

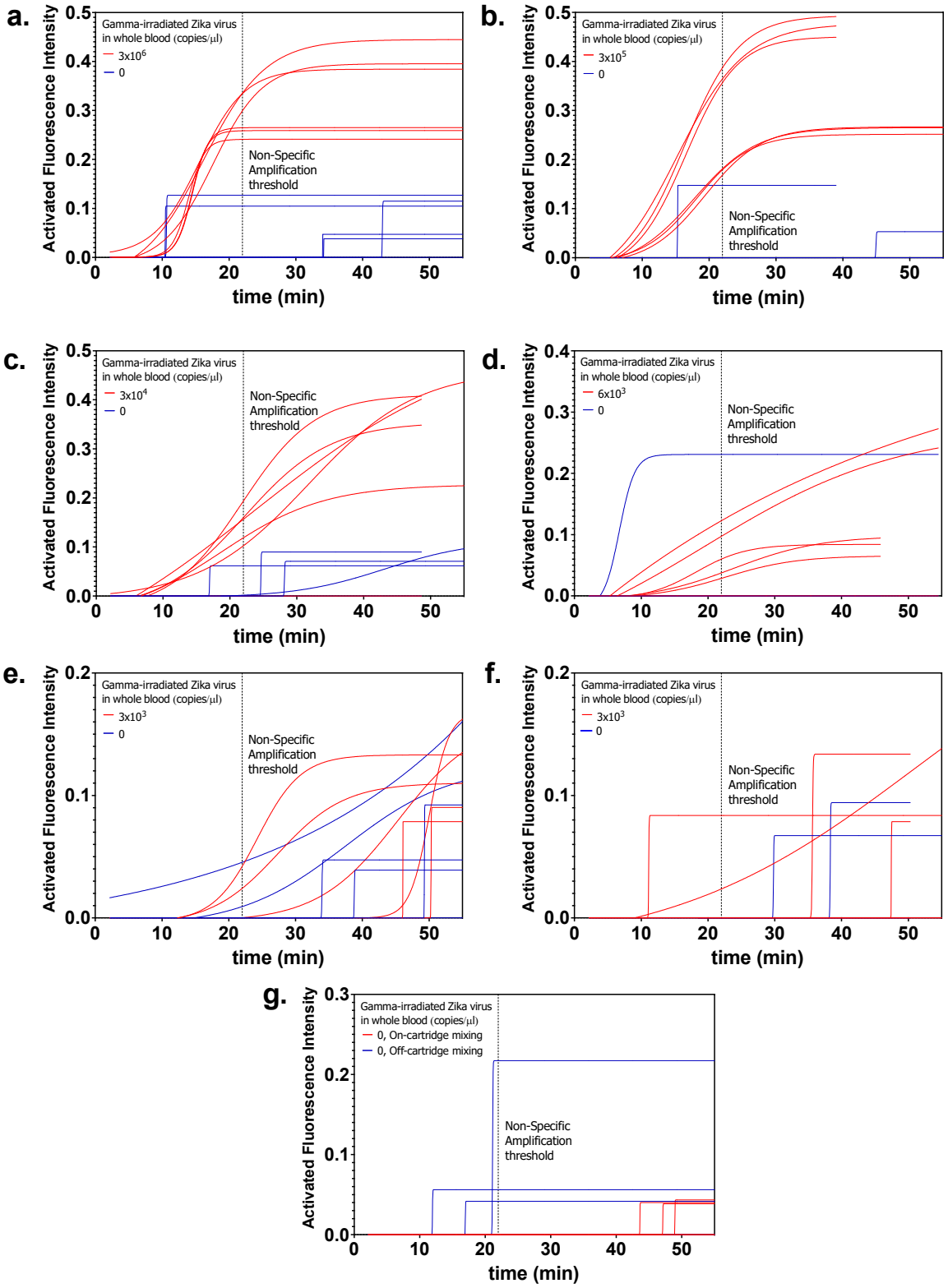

Fig. S12. On-cartridge curve-fitted data from *PathTracker* app for activated intensity analysis. Gamma-irradiated Zika virus in whole blood: **a)** $3x10^6$; **b)** $3x10^5$; **c)** $3x10^4$; **d)** $6x10^3$ (Note: although it appears that the negative sample is amplified early (due to the reflection on the tape), the *PathTracker* app was able to correctly identify this sample); **e)** $3x10^3$; **f)** $1x10^3$; and **g)** 0 (negative control) copies/µl.

Results 1

Given an amplification curve, represented by a sequence of intensity measurements in chronological order $s = \{s[t]\}_{t=1}^{T}$ during T time points. The value of $s[t]$ is obtained either by the activated intensity (AI) or global intensity (GI) scheme. We first define the growth factor as the ratio between the global maximum and minimum of s:

$$\lambda = \frac{\max\limits_{t} s[t]}{\min\limits_{t} s[t]}$$

For the AI, we also apply another constraint on the "speed" of amplification:

$$\gamma = \frac{s[t_{0.9}] - s[t_{0.0}]}{t_{0.9} - t_{0.0}}$$

Where $t_{0.9}$ and $t_{0.0}$ are the indices of the first element belongs to the 0.9-th quantile and the minimum value of the sequence s.

For GB, the amplification curve is classified as *positive* if $\lambda > 4.0$

For AI, the curve is classified as *positive* if $\lambda > 1.2$ and $\gamma > 0.03$

The amplification time for each scheme is defined as follows.

- In AI, we use the time when the first amplified signal appears.
- In GI, we use the time when the activation curve passes the 20% of the global maximum value.

Furthermore, in order to eliminate false positives caused by non-specific amplification, any amplification after 35 minutes or 22 minutes, for GI and AI analysis respectively, were considered non-specific and identified as a negative sample. These times were chosen based on the upper limits of positive amplification combined with looking when known non-specific amplification occurred. Another technique used to prevent false positives was to only acknowledge GI curves when the same compartment also produced a positive AI result.

Video S1. Assembly of Module A.
https://www.youtube.com/watch?v=4ZUZAFaBC9M

Video S2. Sealing of Module B.
https://www.youtube.com/watch?v=gG9coj_RBzM

Video S3. Module A procedures.
https://www.youtube.com/watch?v=T1SVkQ7Q7DA

Color dye solutions were used in this video for better visualization of the mixing processes.

Video S4. Sample loading in Module B.
https://www.youtube.com/watch?v=AfLwon-L85g

The solution transferred to Module B is the solution mixed in **Video S3**.

Video S5. Sample analysis.
https://www.youtube.com/watch?v=3MBmeqKZCXo