

Investigation of the antiviral activity of baicalein towards Zika virus

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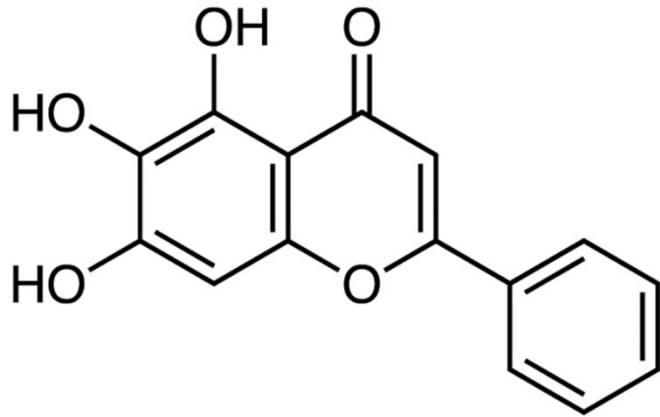
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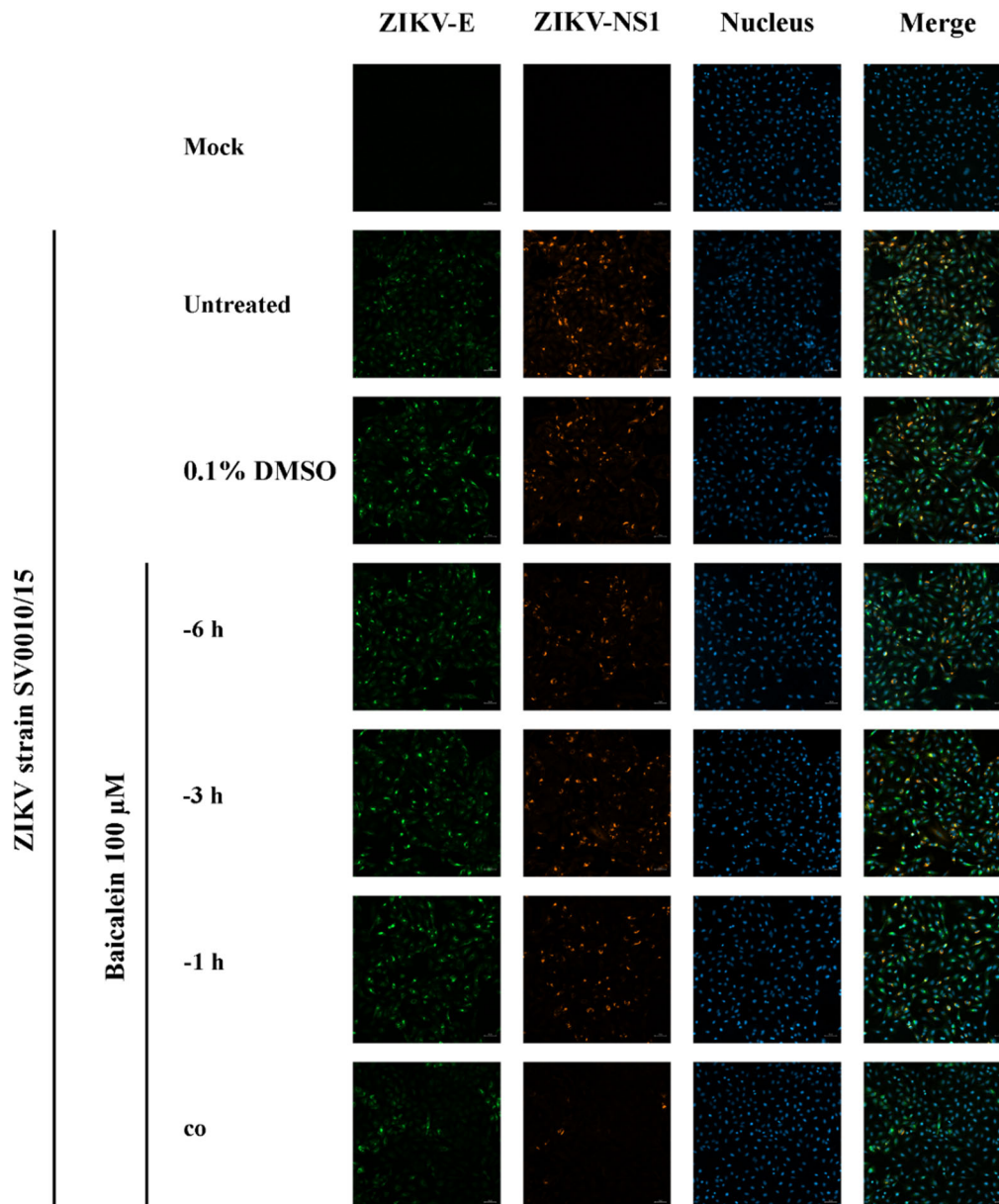
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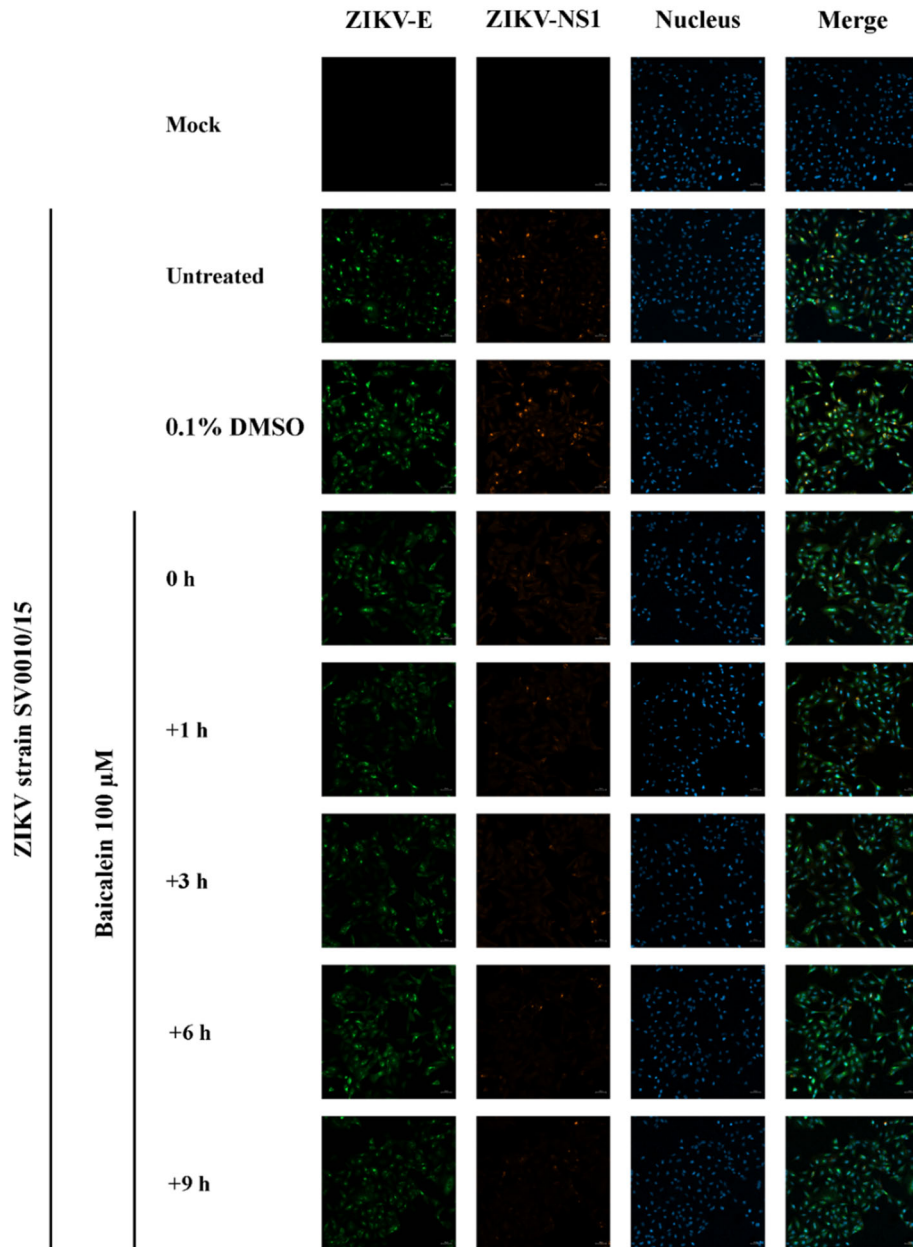
Duncan R. Smith 0000-0002-6592-9852



Supplemental Figure 1. The chemical structure of baicalein (5,6,7-trihydroxyflavone).



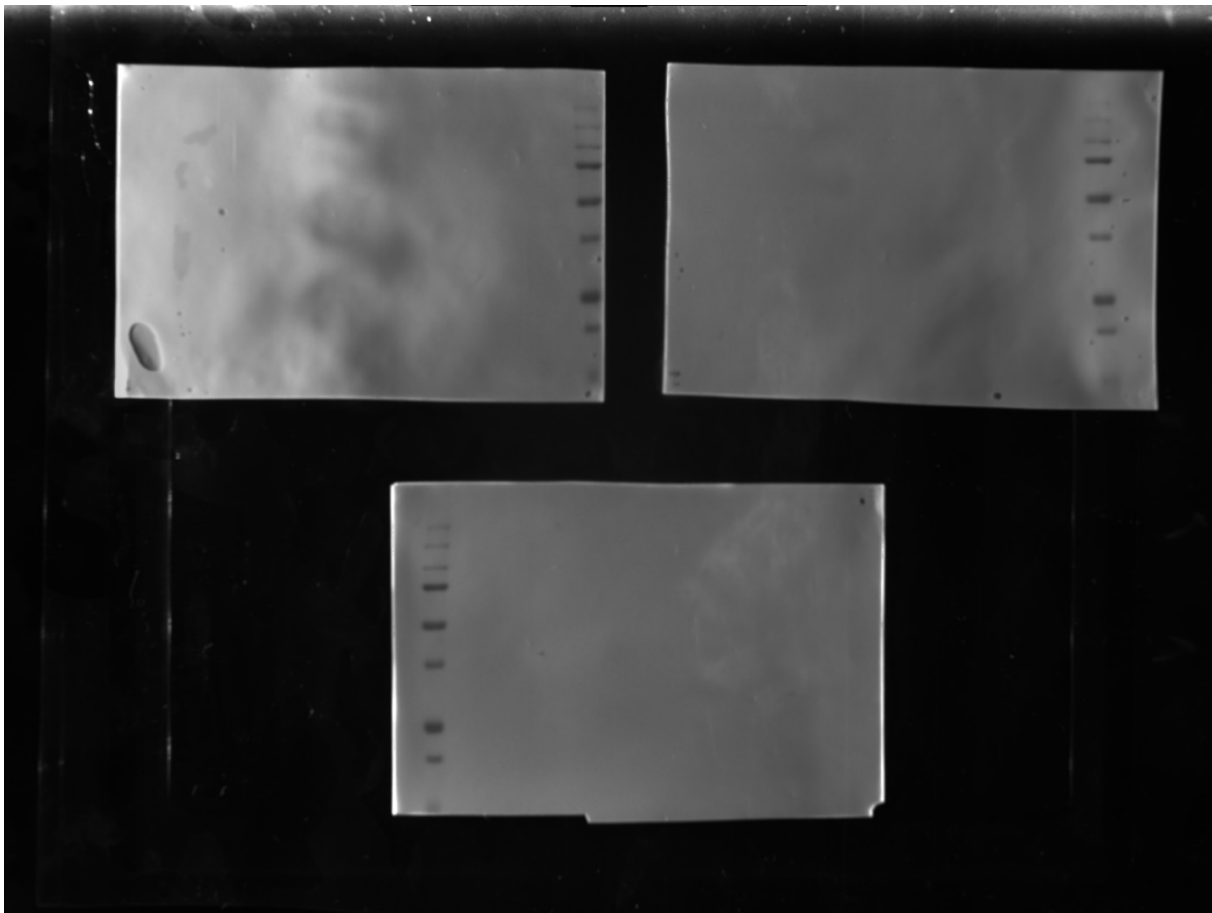
Supplemental Figure 2. Effects of baicalein on viral protein expression at the entry and adsorption stages of ZIKV infection assessed by IFA. A549 cells grown on glass coverslips were pre-treated with 100 μ M baicalein for 1, 3, or 6 h (-1, -3, -6) prior to infection, or cells were co-treated with baicalein during infection. Cells were then infected with MOI 1 of ZIKV. At 24 h post infection, cells were stained with specific antibodies conjugated with fluorescence and DAPI for nuclear staining. Appropriate mock, untreated and DMSO treated controls were undertaken in parallel. Fluorescent signals were detected and visualized under a ZEISS LSM 800 with 100X magnification. Cellular nucleus (blue), viral E protein (green), and viral NS1 protein (red) were shown with non-contrast adjusted un-merged and merged images.

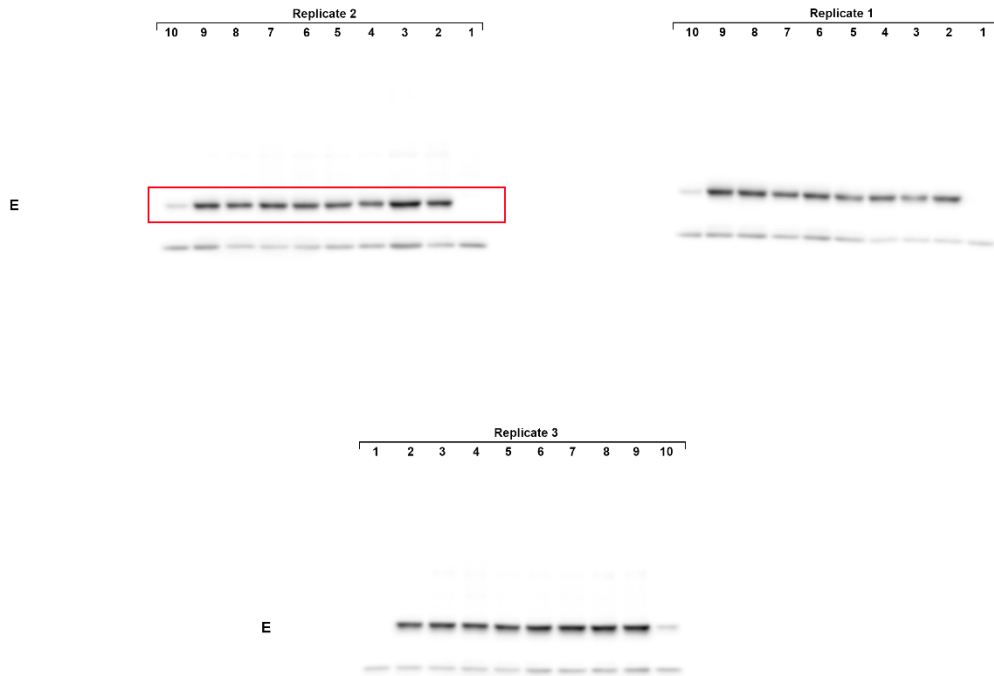


Supplemental Figure 3. Effects of baicalein on viral protein expression at the post-adsorption stage of ZIKV infection assessed by IFA. A549 cells grown on glass coverslips were infected with MOI 1 of ZIKV prior to treating infected cells with 100 μM baicalein at 0, 1, 3, 6, 9 hours (0, +1, +3, +6, +9) after infection. At 24 h post infection, cells were stained with specific antibodies conjugated with fluorescence and DAPI for nuclear staining. Appropriate mock, untreated and DMSO treated controls were undertaken in parallel. Fluorescent signals were detected and visualized under a ZEISS LSM 800 with 100X magnification. Cellular nucleus (blue), viral E protein (green), and viral NS1 protein (red) were shown with non-contrast adjusted un-merged and merged images.

Uncropped western blots

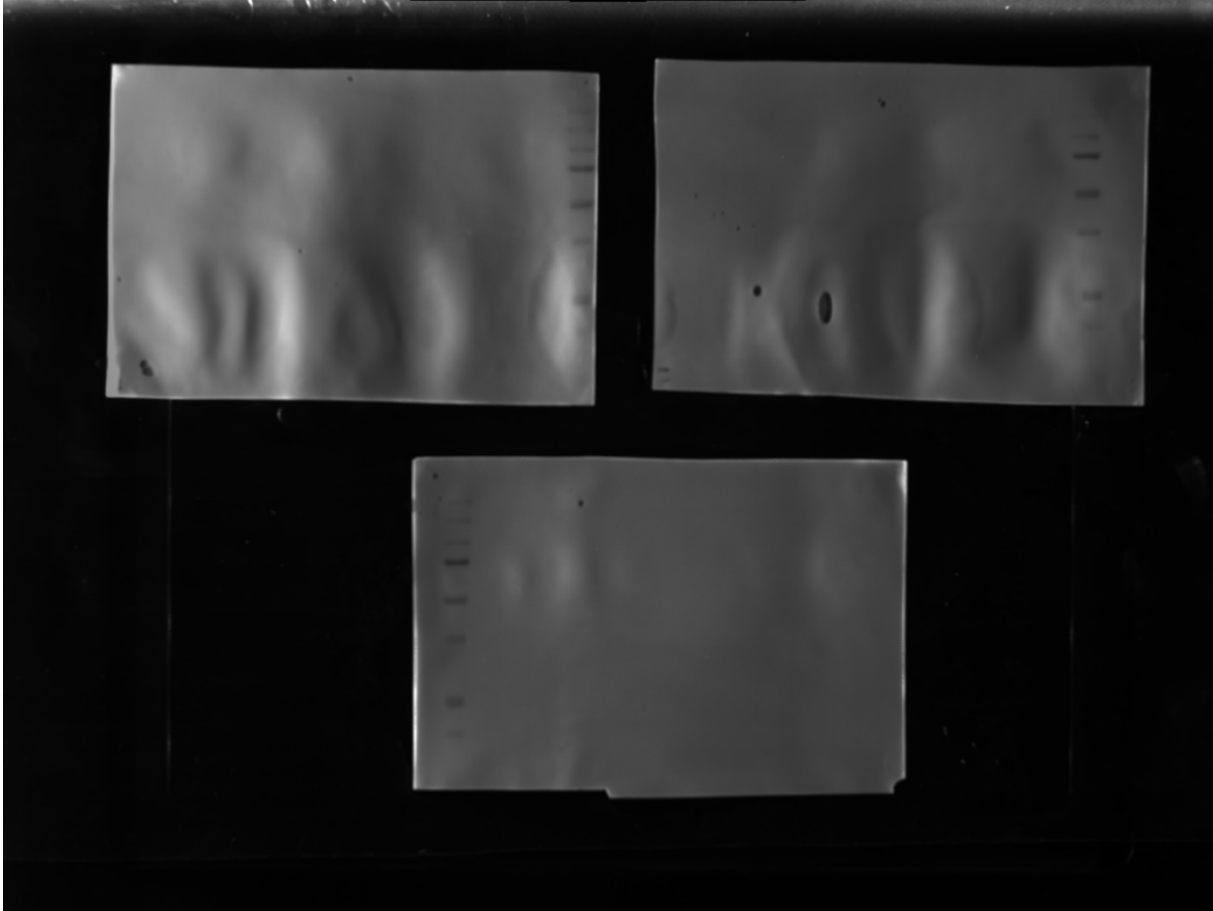
Western blot of viral protein expression at the entry and adsorption stage

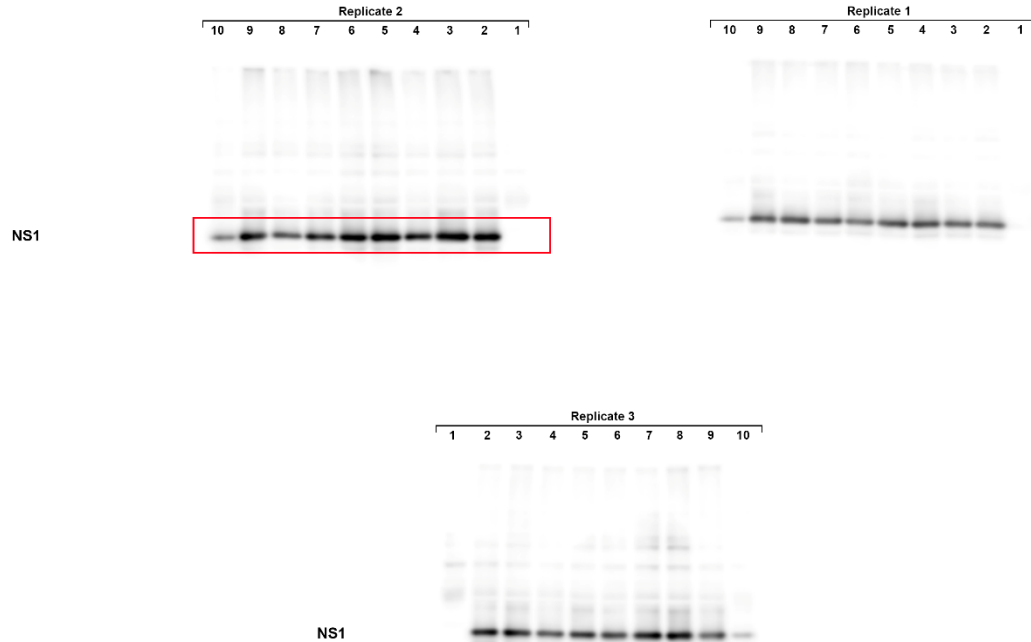




Full western blot membranes of E protein captured by Azure400 (Replicate 1-3), red boxes indicate bands presented in Fig 6; E protein. Replicate 1-2 were flipped horizontally for publication.

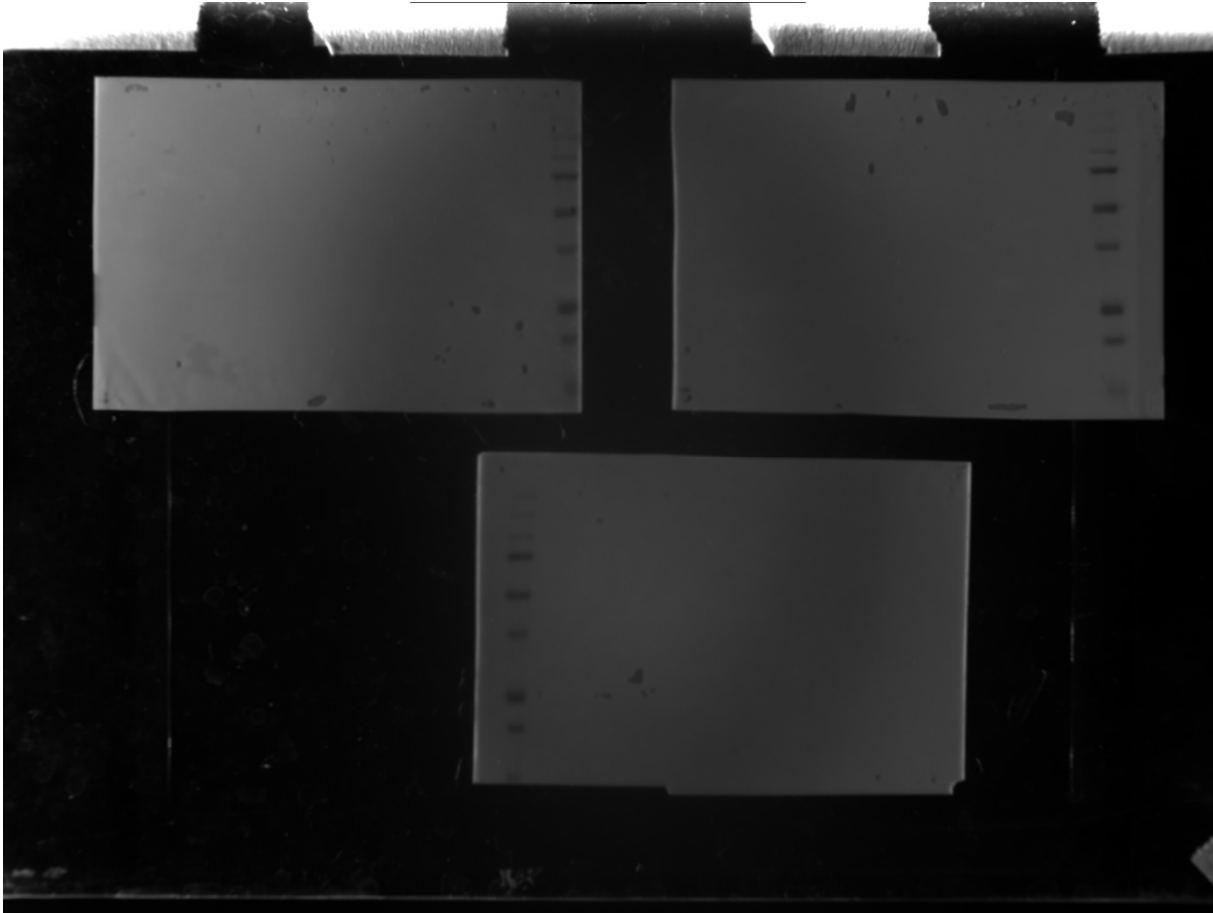
- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO before ZIKV infection for 6 hours
- 4: Treated cells with 0.1% DMSO before ZIKV infection for 3 hours
- 5: Treated cells with 0.1% DMSO before ZIKV infection for 1 hour
- 6: Treated cells with 0.1% DMSO during ZIKV infection
- 7: Treated cells with 100 μ l of Baicalein before ZIKV infection for 6 hours
- 8: Treated cells with 100 μ l of Baicalein before ZIKV infection for 3 hours
- 9: Treated cells with 100 μ l of Baicalein before ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein during ZIKV infection

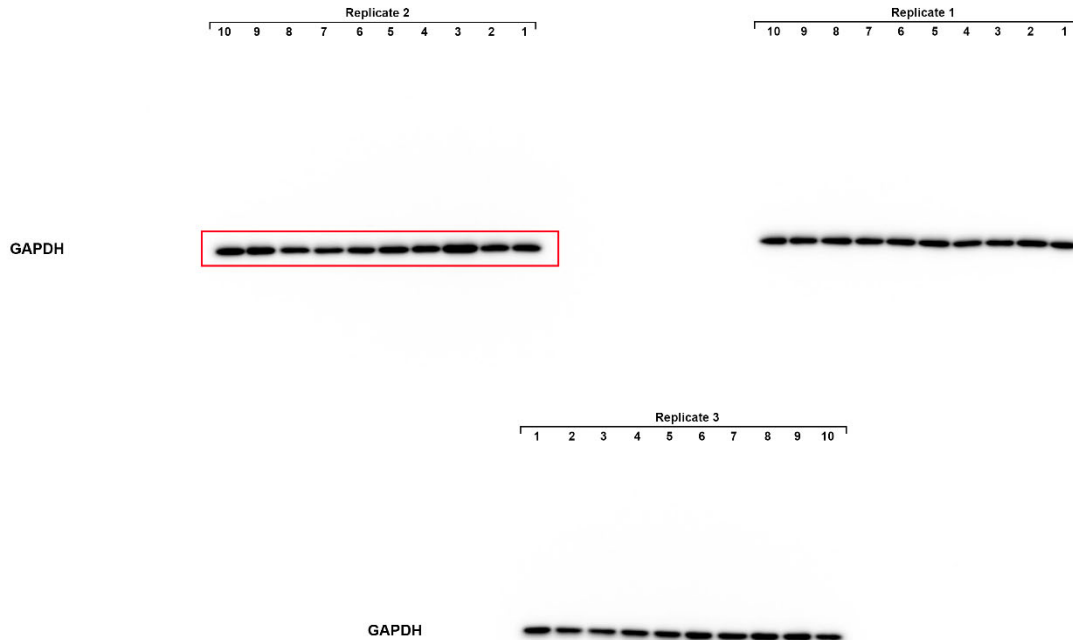




Full western blot membranes of NS1 protein captured by Azure400 (Rep 1-3), red boxes indicate bands presented in Fig 6; NS1 protein. Replicate 1-2 were flipped horizontally for publication.

- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO before ZIKV infection for 6 hours
- 4: Treated cells with 0.1% DMSO before ZIKV infection for 3 hours
- 5: Treated cells with 0.1% DMSO before ZIKV infection for 1 hour
- 6: Treated cells with 0.1% DMSO during ZIKV infection
- 7: Treated cells with 100 μ l of Baicalein before ZIKV infection for 6 hours
- 8: Treated cells with 100 μ l of Baicalein before ZIKV infection for 3 hours
- 9: Treated cells with 100 μ l of Baicalein before ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein during ZIKV infection

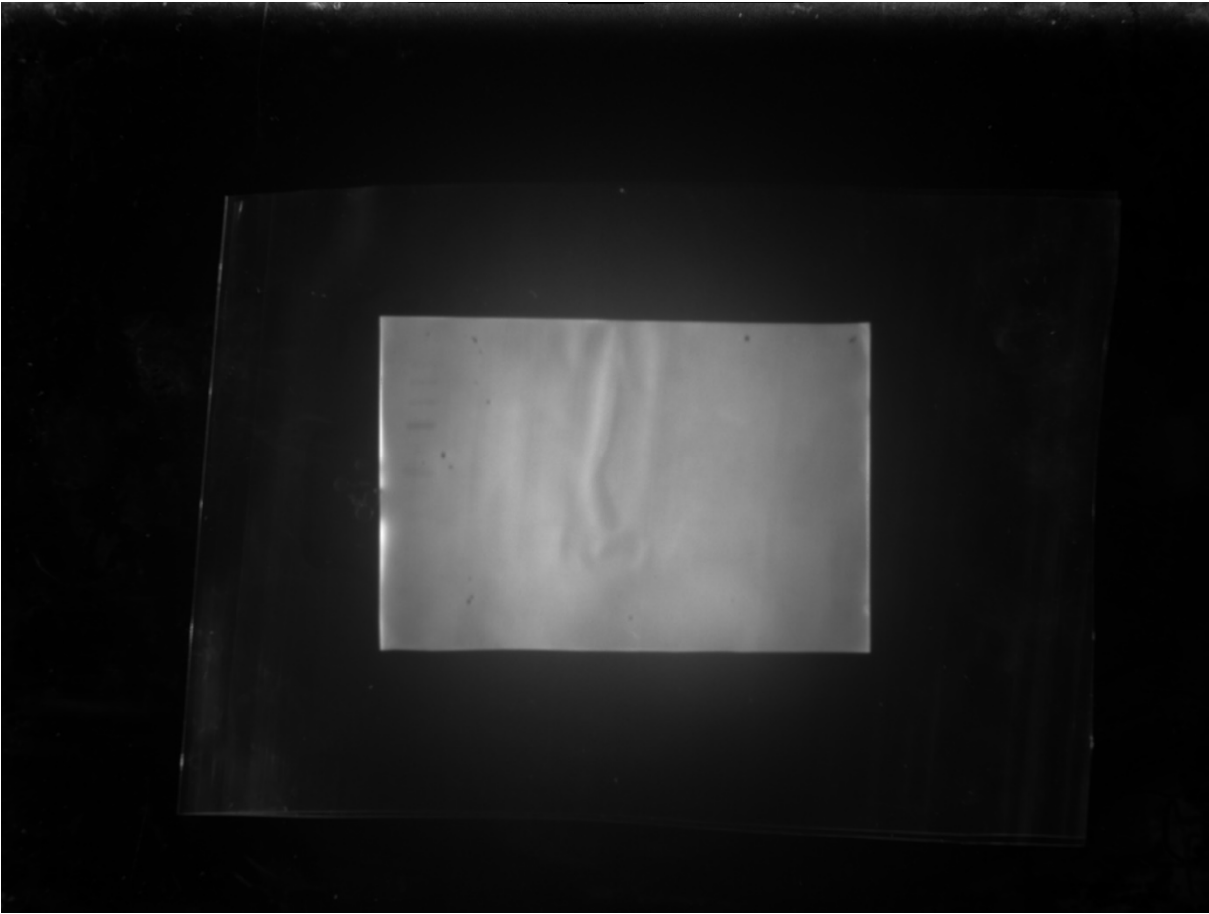


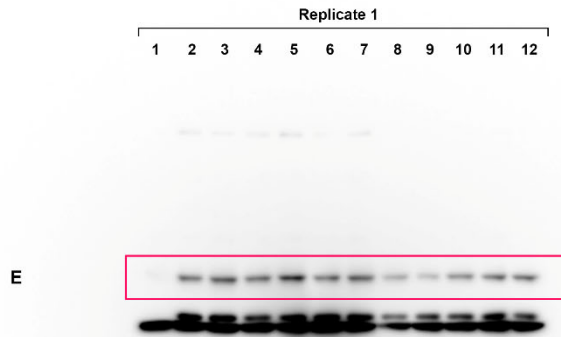


Full western blot membranes of GAPDH captured by Azure400 (Rep 1-3), red boxes indicate bands presented in Fig 6; GAPDH. Replicate 1-2 were flipped horizontally for publication.

- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO before ZIKV infection for 6 hours
- 4: Treated cells with 0.1% DMSO before ZIKV infection for 3 hours
- 5: Treated cells with 0.1% DMSO before ZIKV infection for 1 hour
- 6: Treated cells with 0.1% DMSO during ZIKV infection
- 7: Treated cells with 100 μ l of Baicalein before ZIKV infection for 6 hours
- 8: Treated cells with 100 μ l of Baicalein before ZIKV infection for 3 hours
- 9: Treated cells with 100 μ l of Baicalein before ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein during ZIKV infection

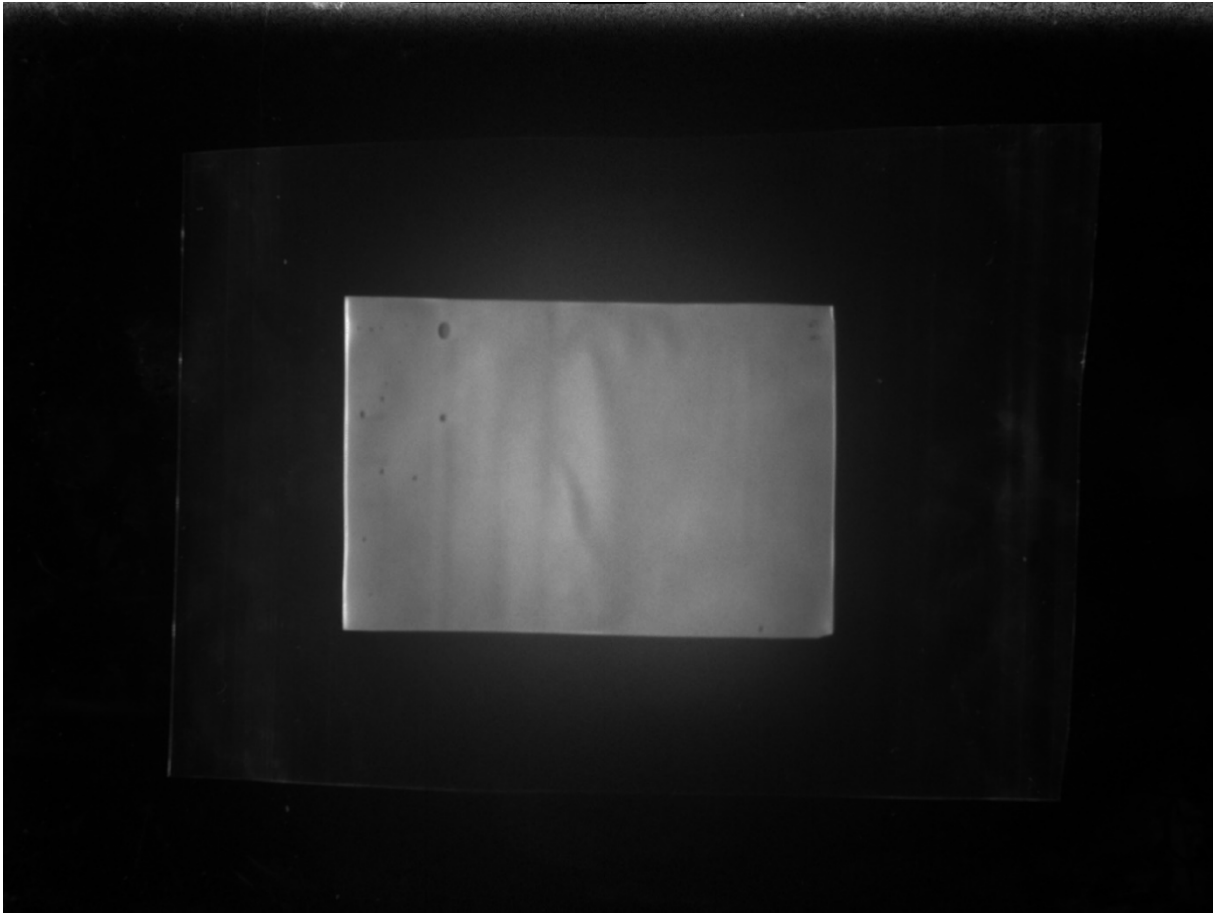
Western blot of viral protein expression at the post-adsorption stage

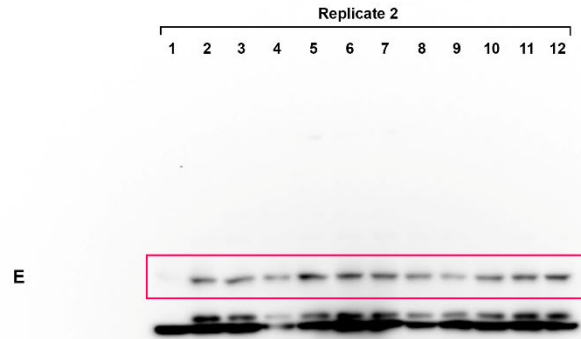




Full western blot membranes of E protein captured by Azure400 (Replicate 1), red boxes indicate bands presented in Fig 8; E protein.

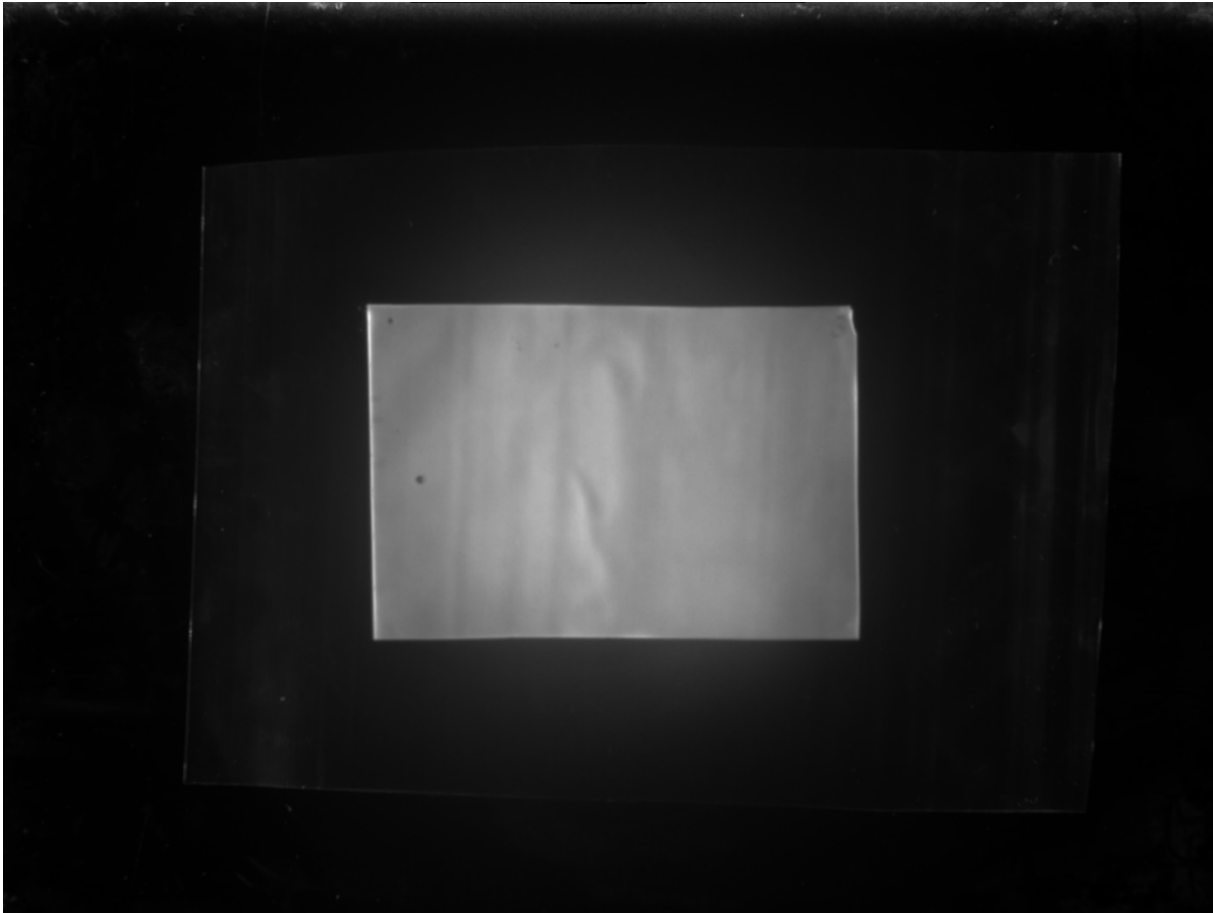
- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours

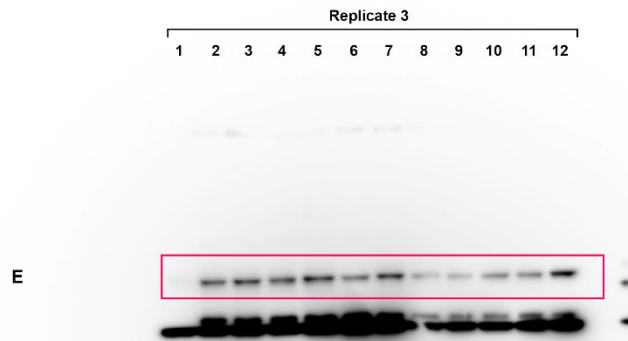




Full western blot membranes of E protein captured by Azure400 (Replicate 2), red boxes indicate bands presented in Fig 8; E protein.

- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours





Full western blot membranes of E protein captured by Azure400 (Replicate 3), red boxes indicate bands presented in Fig 8; E protein.

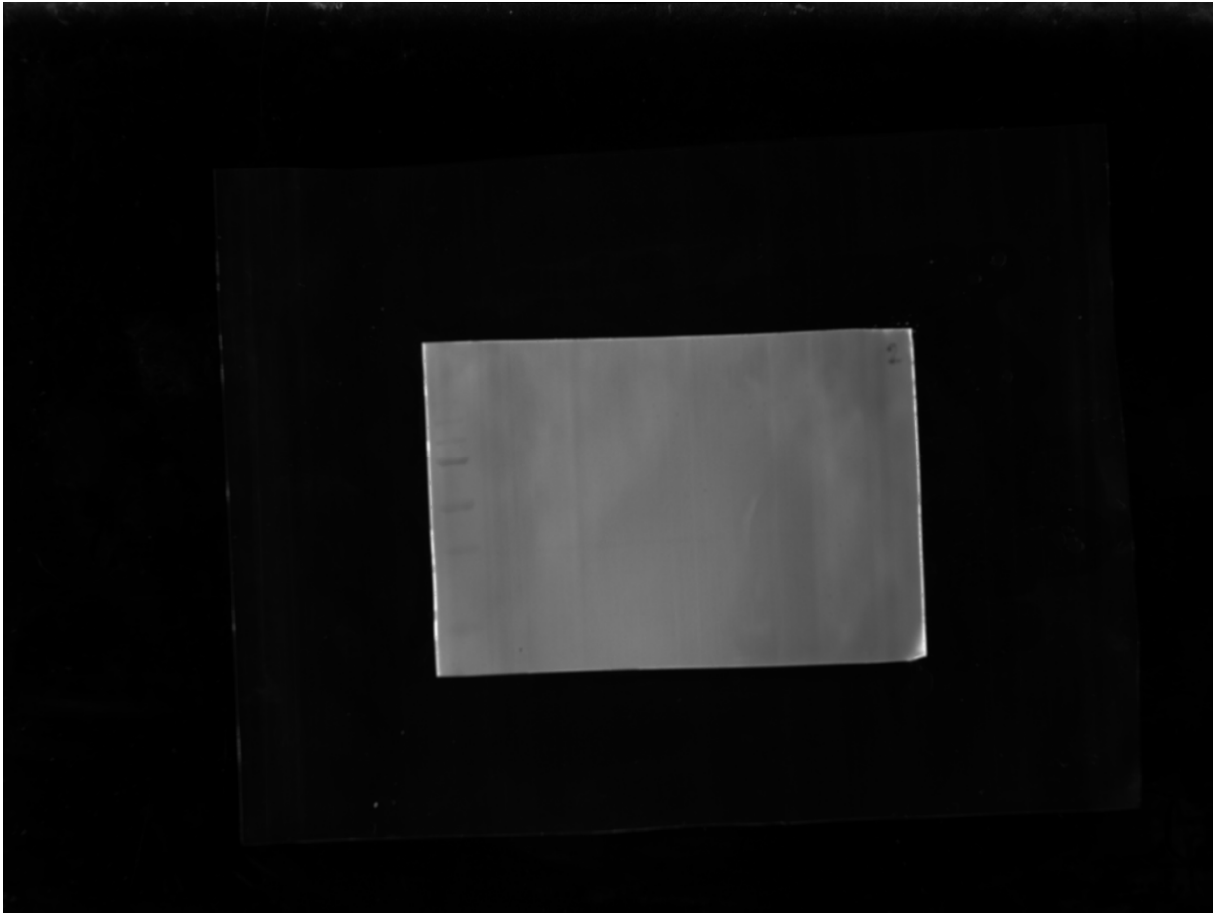
- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours

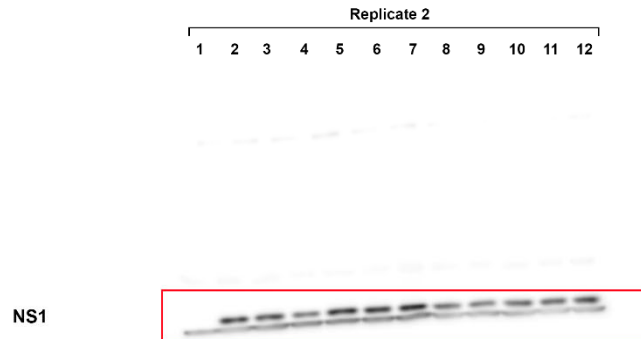




Full western blot membranes of NS1 protein captured by Azure400 (Rep 1), red boxes indicate bands presented in Fig 8; NS1 protein.

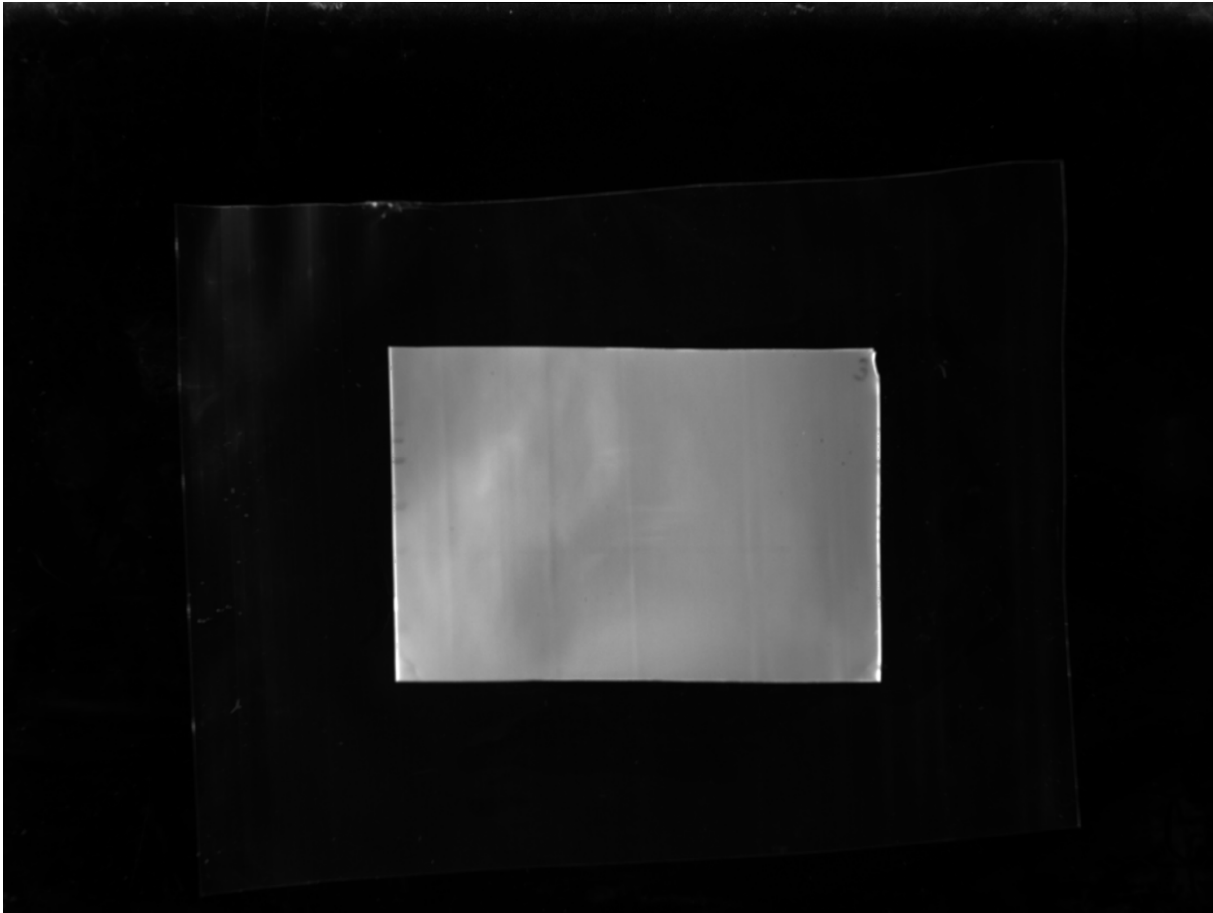
- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours





Full western blot membranes of NS1 protein captured by Azure400 (Rep 2), red boxes indicate bands presented in Fig 8; NS1 protein.

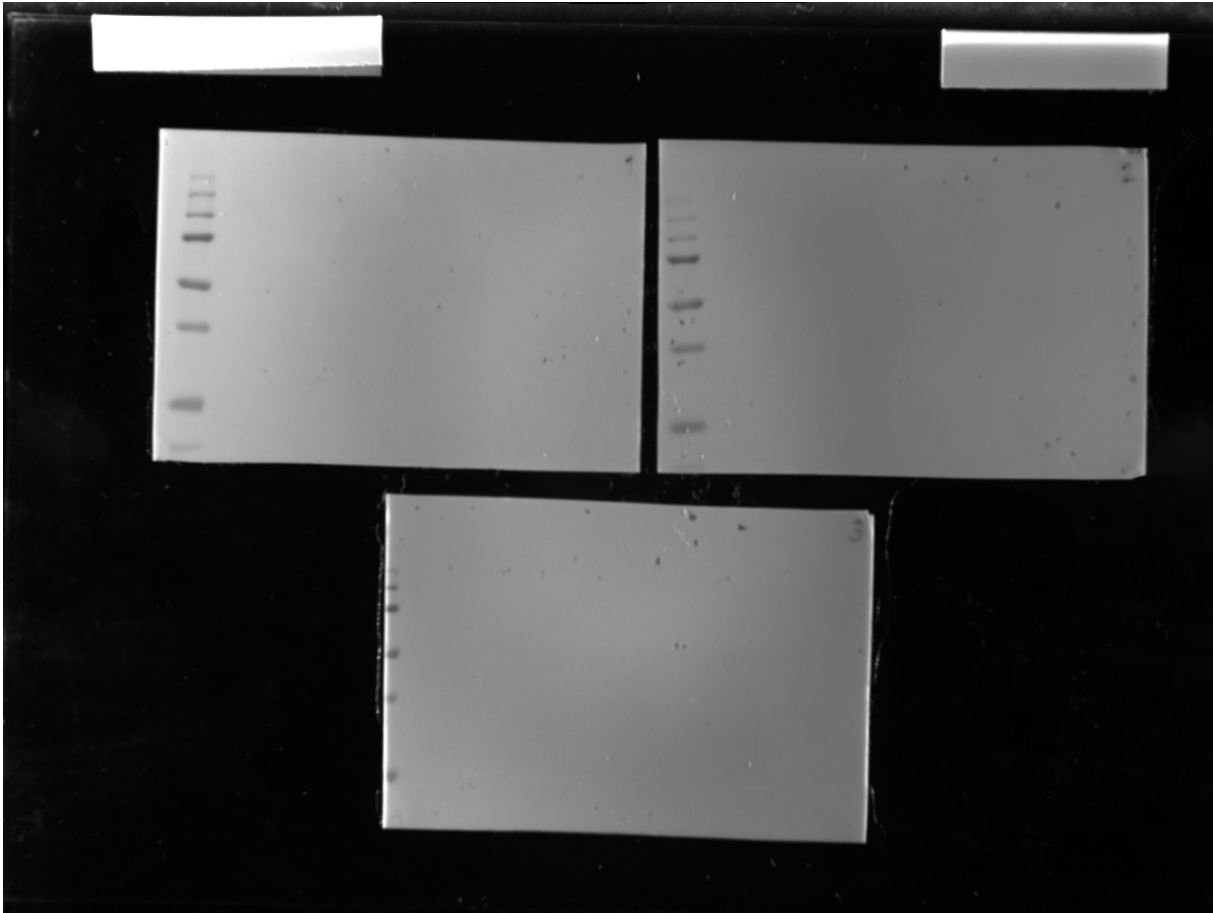
- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours

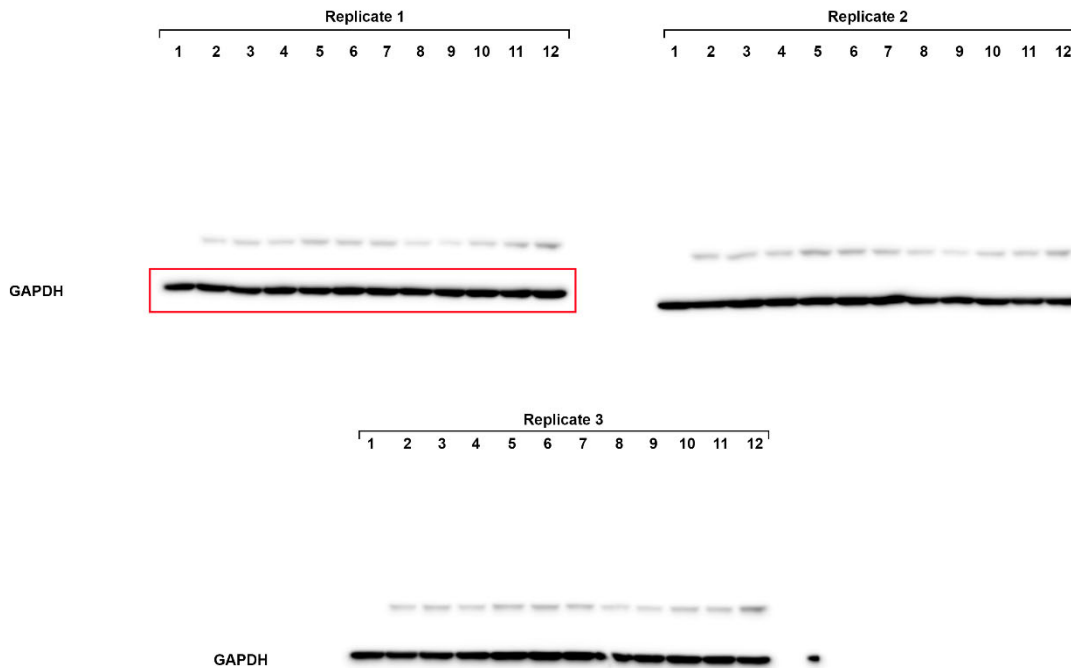




Full western blot membranes of NS1 protein captured by Azure400 (Rep 3), red boxes indicate bands presented in Fig 8; NS1 protein.

- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours





Full western blot membranes of GAPDH captured by Azure400 (Rep 1-3), red boxes indicate bands presented in Fig 8; GAPDH.

- 1: Mock
- 2: Untreated cells with ZIKV infection
- 3: Treated cells with 0.1% DMSO after ZIKV infection for 0 hour
- 4: Treated cells with 0.1% DMSO after ZIKV infection for 1 hour
- 5: Treated cells with 0.1% DMSO after ZIKV infection for 3 hours
- 6: Treated cells with 0.1% DMSO after ZIKV infection for 6 hours
- 7: Treated cells with 0.1% DMSO after ZIKV infection for 9 hours
- 8: Treated cells with 100 μ l of Baicalein after ZIKV infection for 0 hour
- 9: Treated cells with 100 μ l of Baicalein after ZIKV infection for 1 hour
- 10: Treated cells with 100 μ l of Baicalein after ZIKV infection for 3 hours
- 11: Treated cells with 100 μ l of Baicalein after ZIKV infection for 6 hours
- 12: Treated cells with 100 μ l of Baicalein after ZIKV infection for 9 hours