

The Antiviral Drug Arbidol Inhibits Zika Virus

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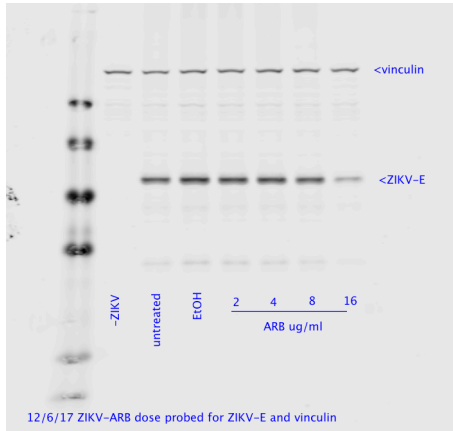
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SUPPLEMENTAL MATERIAL

Original Western Blot Images for Figures 2, 5, and 7

Figure 2 Original Blot Images

First probing: Vinculin and ZIKV E



Second probing: ZIKV NS1 and Capsid

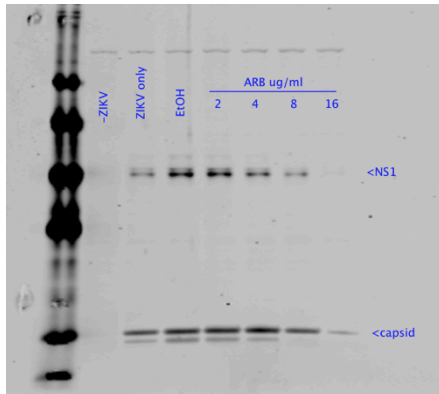
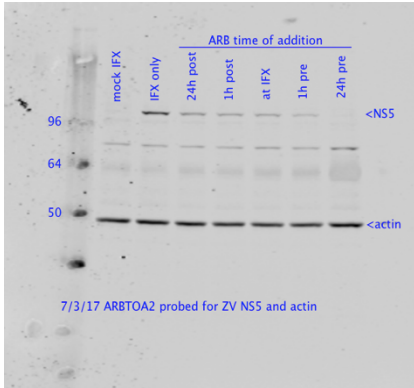


Figure 5 Original Blot Images

First probing: ZIKV NS5 & Actin



Second probing: ZIKV NS1

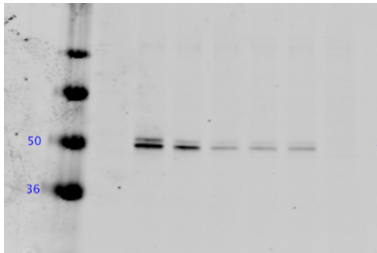


Figure 7A Original Blot Images

Fig 7A: Blot 1 probing: Vinculin and ZIKV NS1

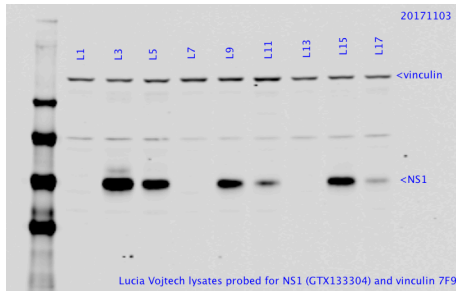


Fig 7A: Blot 2 first probing: ZIKV E

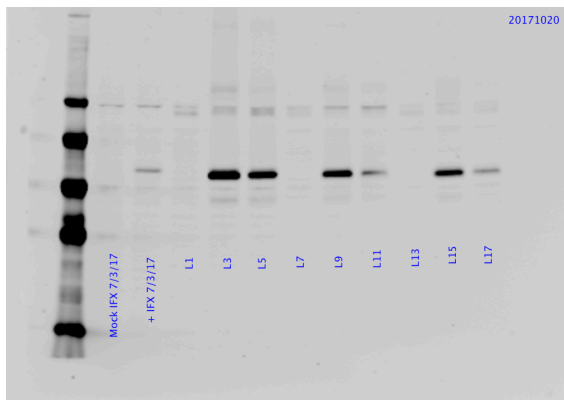


Fig 7A: Blot 2 second probing: Vinculin and ZIKV Capsid

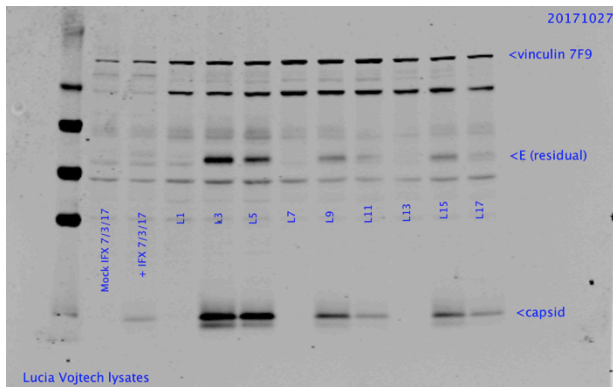
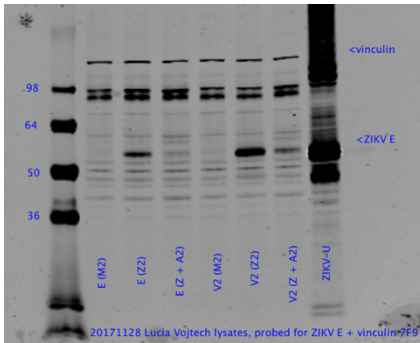
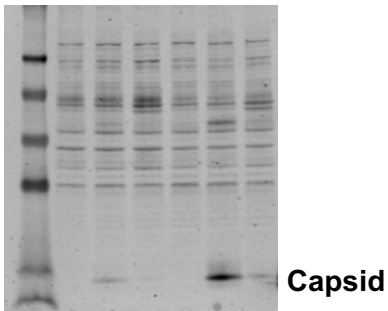


Figure 7D Original Blot Images

First probing: ZIKV E and Vinculin



Second probing: ZIKV Capsid



Supplemental Figures

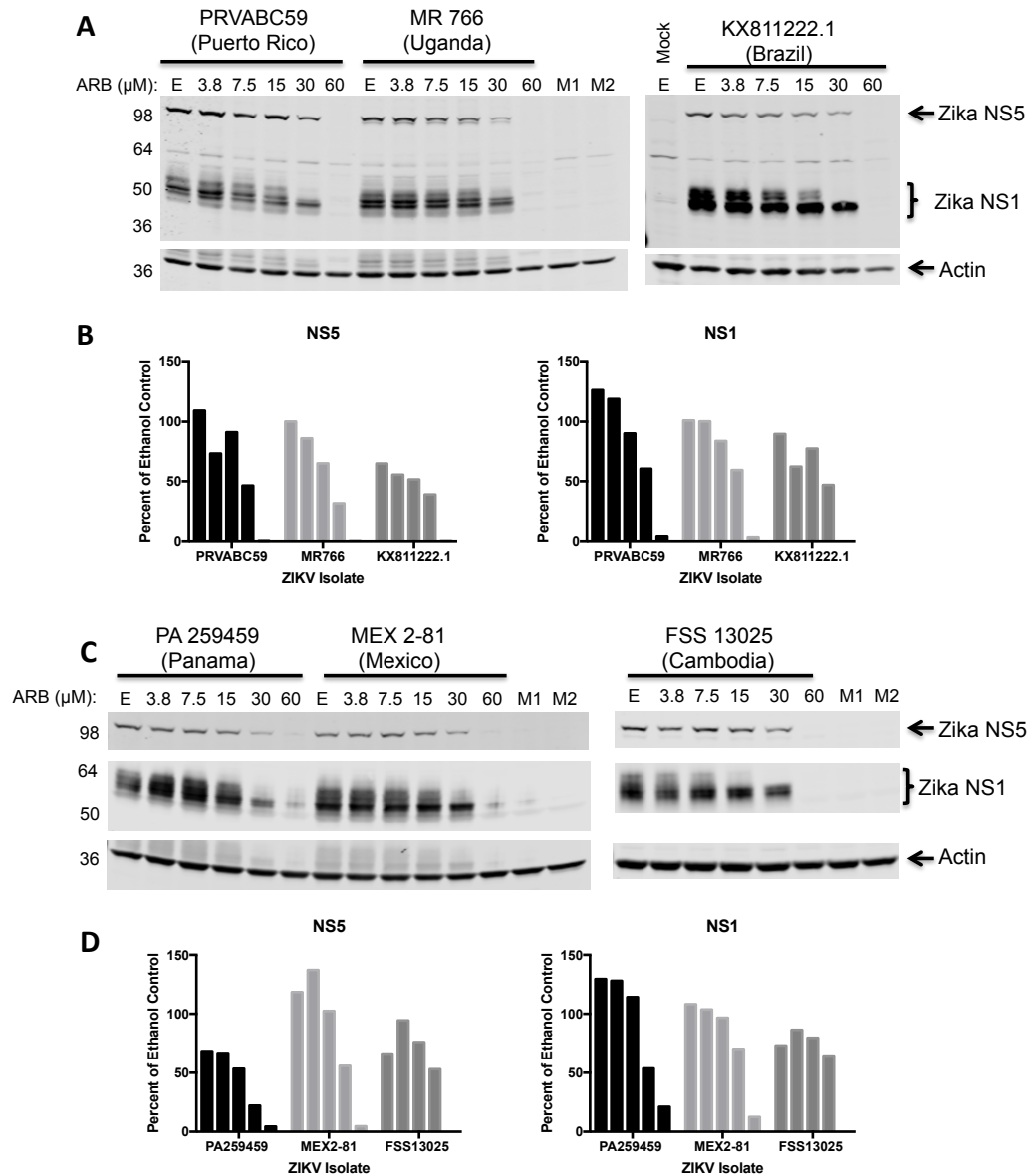


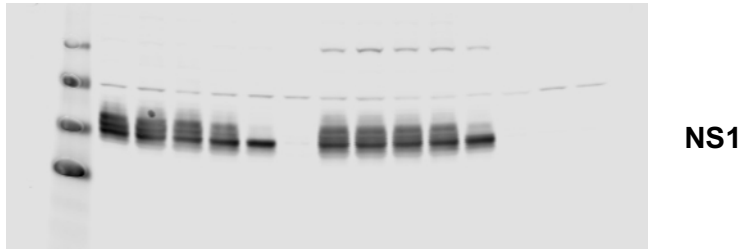
Figure S1 – ARB inhibits an African and multiple Asian ZIKV isolates. Vero cells were treated with ethanol solvent control (E) or 3.8, 7.5, 15, 30, or 60 μM of ARB for 30 minutes prior to infection with the indicated isolates of ZIKV at an MOI of 0.01. M1 = mock infected cells treated with ethanol solvent control. M2 = mock infected cells treated

with 60 μ M ARB. Molecular weight markers are as indicated. Panel A, ARB suppression of Puerto Rico, Uganda, and Brazil virus isolates. Panel C, ARB suppression of Panama, Mexico, and Cambodia virus isolates. Panels B and D, bar graphs below each blot show relative pixel intensities across the 5 doses of ARB for each virus isolate. The images represent composites of the following antibody probings: PRVABC59 and MR766 samples were run on the same gel and the blot was first probed with rabbit anti-ZIKV-NS1 (detected with anti-rabbit 800 labeled secondary antibodies), stripped and probed a second time with rabbit anti-ZIKV-NS5 antiserum (detected with anti-rabbit 680 labeled secondary antibodies), and stripped and probed a third time with goat anti-actin (detected with anti-goat 800 labeled secondary antibodies). PA259459 and MEX2-81 samples were run on the same gel the blot was first probed with rabbit anti-ZIKV-NS1 (detected with anti-rabbit 800 labeled secondary antibodies) and goat anti-actin (detected with anti-goat 680 labeled secondary antibodies). The blot was stripped and probed with rabbit anti-ZIKV-NS5 antiserum (detected with anti-rabbit 680 labeled secondary antibodies). FSS13025 samples were first probed with rabbit anti-ZIKV-NS5 (detected with anti-rabbit 800 labeled secondary antibodies) and goat anti-actin (detected with anti-goat 680 labeled secondary antibodies). The blot was stripped and probed with rabbit anti-ZIKV-NS1 antiserum (detected with anti-rabbit 680 labeled secondary antibodies). KX811222.1 samples were probed with a triplex of rabbit anti-NS5 and NS5 antiserum (detected with anti-rabbit 680 labeled secondary antibodies) plus goat anti-actin (detected with anti-goat 800 labeled secondary antibodies). Original blot images are found below.

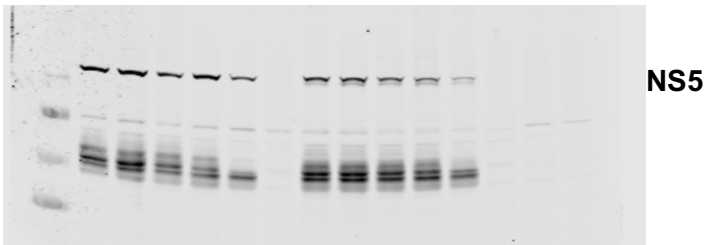
Original Blot Images for Figure S1

PRVABC59 (left), MR766 (right)

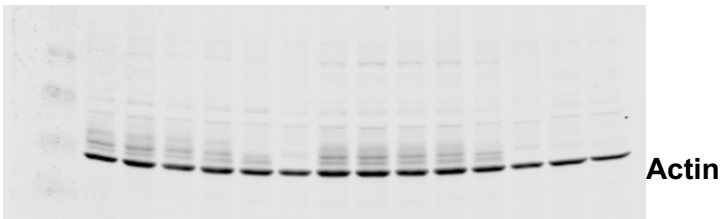
First probing: ZIKV NS1



Second probing: ZIKV NS5

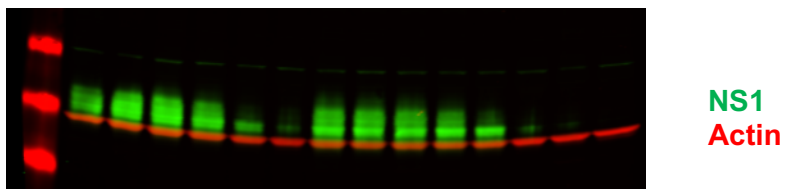


Third probing: actin

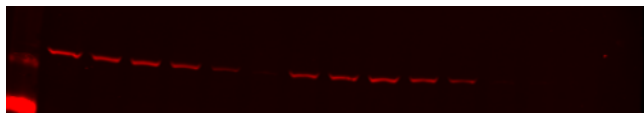


PA259459 (left), MEX2-81 (right)

First probing: ZIKV NS1 and actin



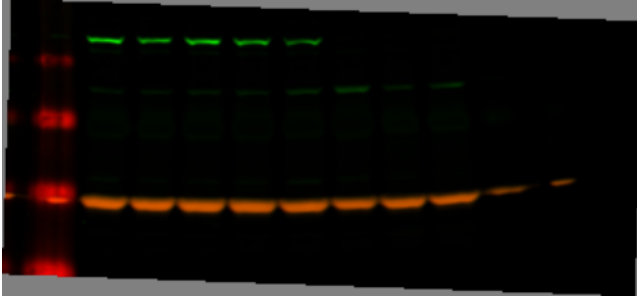
Second probing: ZIKV NS5



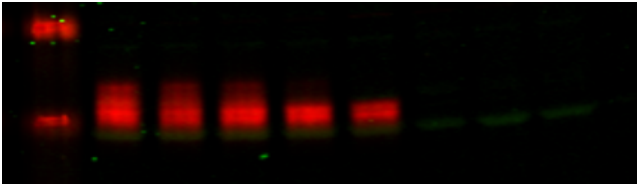
Original Blot Images for Figure S1

FSS13025

First probing: ZIKV NS5, actin

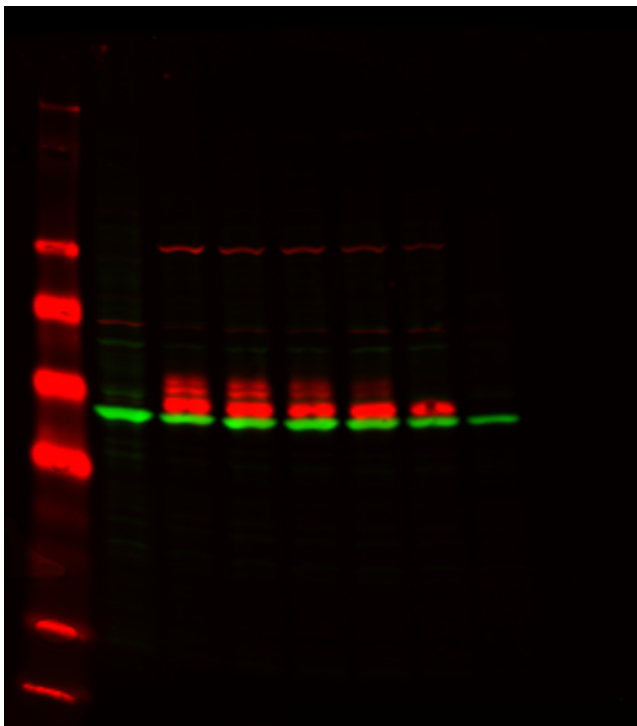


Second probing: ZIKV Capsid



KX811222.1

First probing: ZIKV NS5, ZIKV NS1, and actin



NS5

NS1
Actin

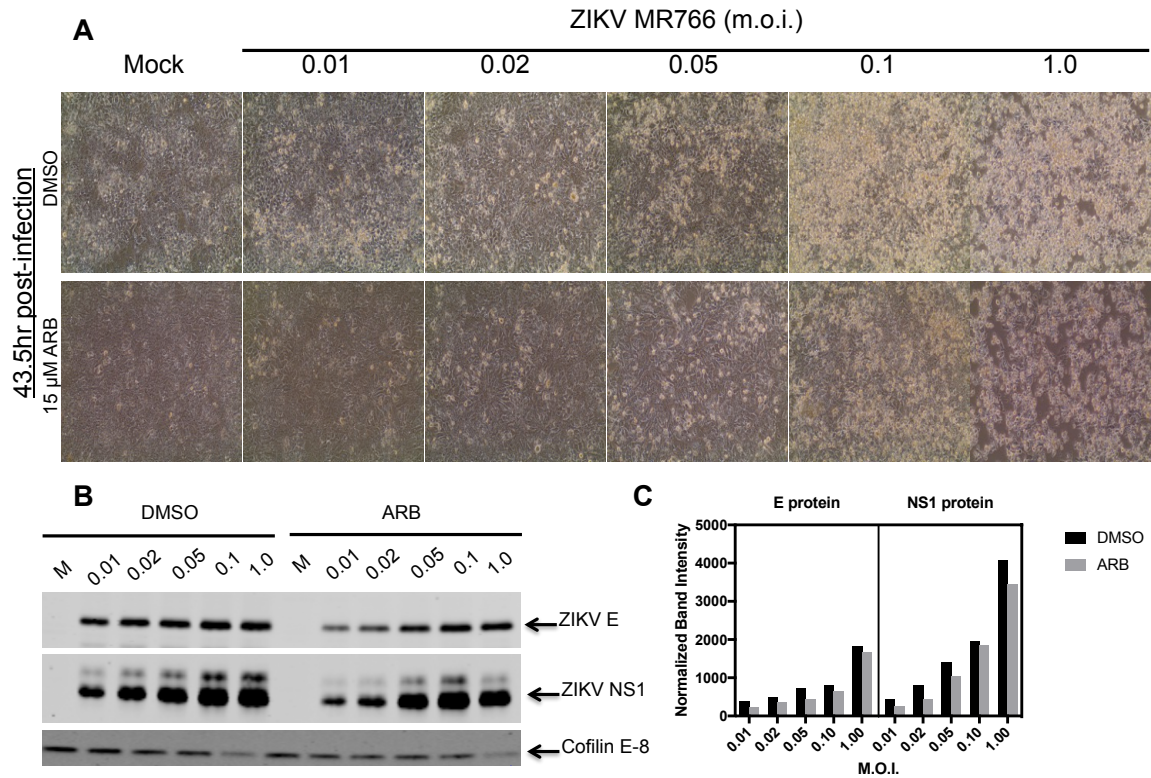
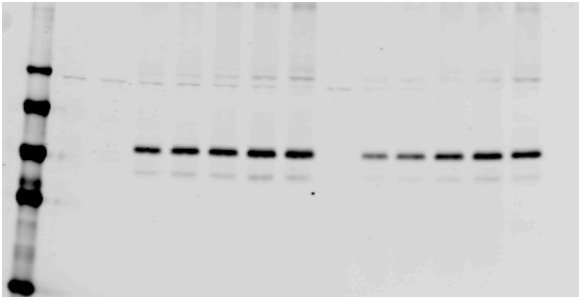


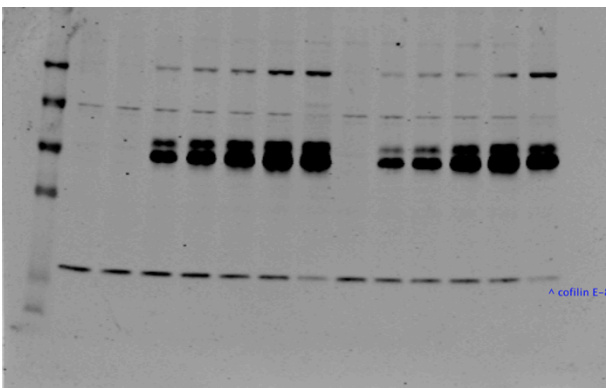
Figure S2 – ARB inhibits ZIKV replication and virus-induced CPE in human hepatoma Huh7.5.1 cells. Huh7.5.1 cells were infected at the indicated MOIs with ZIKV MR766 for 1.5 hours. Viral inocula were removed, cells were washed with PBS, and fresh media containing DMSO solvent control or 15 μ M ARB was added to cells. A, light microscopy of mock and ZIKV-infected cultures. B, western blot of ZIKV E and NS1 and cellular protein Cofilin E-8 expression. The image is a composite of the following antibody probings: the blot was first probed with rabbit anti-ZIKV-E (detected with anti-rabbit 800 labeled secondary antibodies). The blot was stripped and reprobed with mouse anti-Cofilin E-8 (detected with anti-mouse 680 labeled secondary antibodies) and rabbit anti-ZIKV-NS1 antiserum (detected with anti-rabbit 800 labeled secondary antibodies). C, Normalized band intensity of western blot data. Original blot images are shown below.

Original Blot Images for Figure S2

First probing: ZIKV E



Second probing: ZIKV NS1 and cofilin E-8



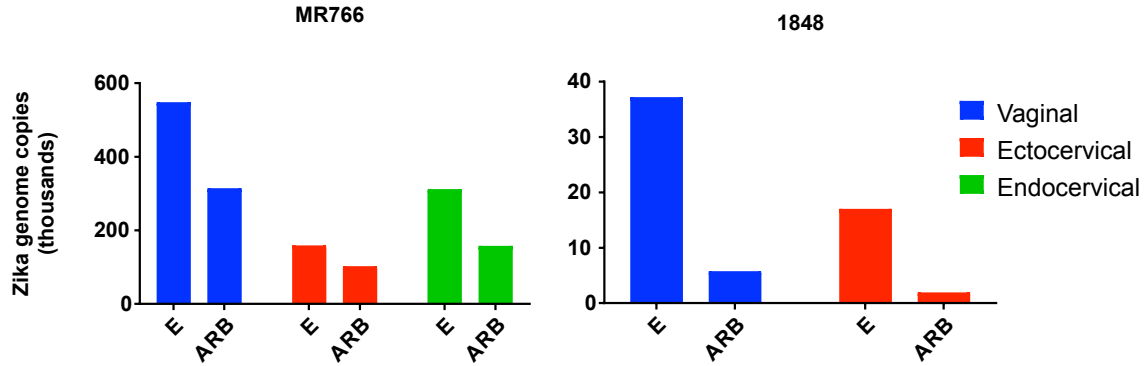


Figure S3: ARB inhibits ZIKV RNA accumulation in primary human vaginal and cervical epithelial cells. Cells were cultured as described in the Material and Methods and infected with ZIKV strains MR766, an African lineage isolate, or 1848, an Asian lineage isolate, at a MOI of 1 for 72 hours, in the presence and absence of 20 μ M ARB. RNA was extracted, reverse-transcribed and ZIKV genomes measured by droplet digital PCR.