Attenuation of Zika Virus by Passage in Human HeLa Cells

Li Li 1, Natalie D. Collins 2,3, Steven G. Widen 4, Emily H. Davis 1, Jaclyn A. Kaiser 2, Melodee M. White 5, M. Banks Greenberg 3, Alan D.T. Barrett 1,6,7, Nigel Bourne 2,5,6,7, Vanessa V. Sarathy 1,6,7,*

1 Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555, USA; lili@utmb.edu (L.L.);
emidavis@utmb.edu (E.H.D.); abarrett@utmb.edu (A.D.T.B.)
2 Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, USA;
natalie.d.collins.mil@mail.mil (N.D.C.); jakaiser@utmb.edu (J.A.K.); nibourne@utmb.edu (N.B.)
3 Viral Disease Branch, Walter Reed Army Institute for Research, Silver Spring, MD 20910, USA
4 Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555,
USA; sgwiden@utmb.edu (S.G.W.)
5 Department of Pediatrics, University of Texas Medical Branch, Galveston, TX 77555, USA; m3white@utmb.edu
(M.M.W.); mbgreenb@utmb.edu (M.B.G.)
6 Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX 77555, USA
7 Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX 77555, USA

* Correspondence: vvsarath@utmb.edu; Tel.: 01-409-747-2403
**Figure S1.** Passaging of ZIKV in HeLa cells attenuates cytokine production in A549 epithelial cells. Secreted cytokine levels from A549 cells infected with WT ZIKV, HeLa-P4, HeLa-P5, or mock at 1, 2, or 3 days p.i were quantified from 50 microliter samples (n = 2). (a) Cytokine and chemokine levels increased on day 1, 2, or 3 following infection with HeLa-P4 and HeLa-P5 but not with WT ZIKV; (b) Cytokines and chemokines increased by infection with WT ZIKV on day 2 p.i. or (c) on days 2 and 3 p.i. Not shown: IL-1β, IL-4, IL-12p70, and IL-13 were detected at very low levels (< 2 pg/mL); IL-1RA and IL-10 were not detected in any samples. Kruskal-Wallis tests with Dunn’s multiple comparisons were used to compare WT ZIKV, HeLa-P4, and HeLa-P5 to mock and to compare mock, HeLa-P4, and HeLa-P5 to WT ZIKV. Error bars represent standard deviation. *p < 0.05 WT ZIKV compared to mock; ∞ p < 0.05 WT ZIKV compared to HeLa-P4 or HeLa-P5. No differences were detected between HeLa-P4 and HeLa-P5 induction of cytokines.