Supporting Information for

Original article

In vitro and *in vivo* characterization of erythrosin B and derivatives against Zika virus

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Received 23 August 2021; received in revised form 30 September 2021; accepted 4 October 2021

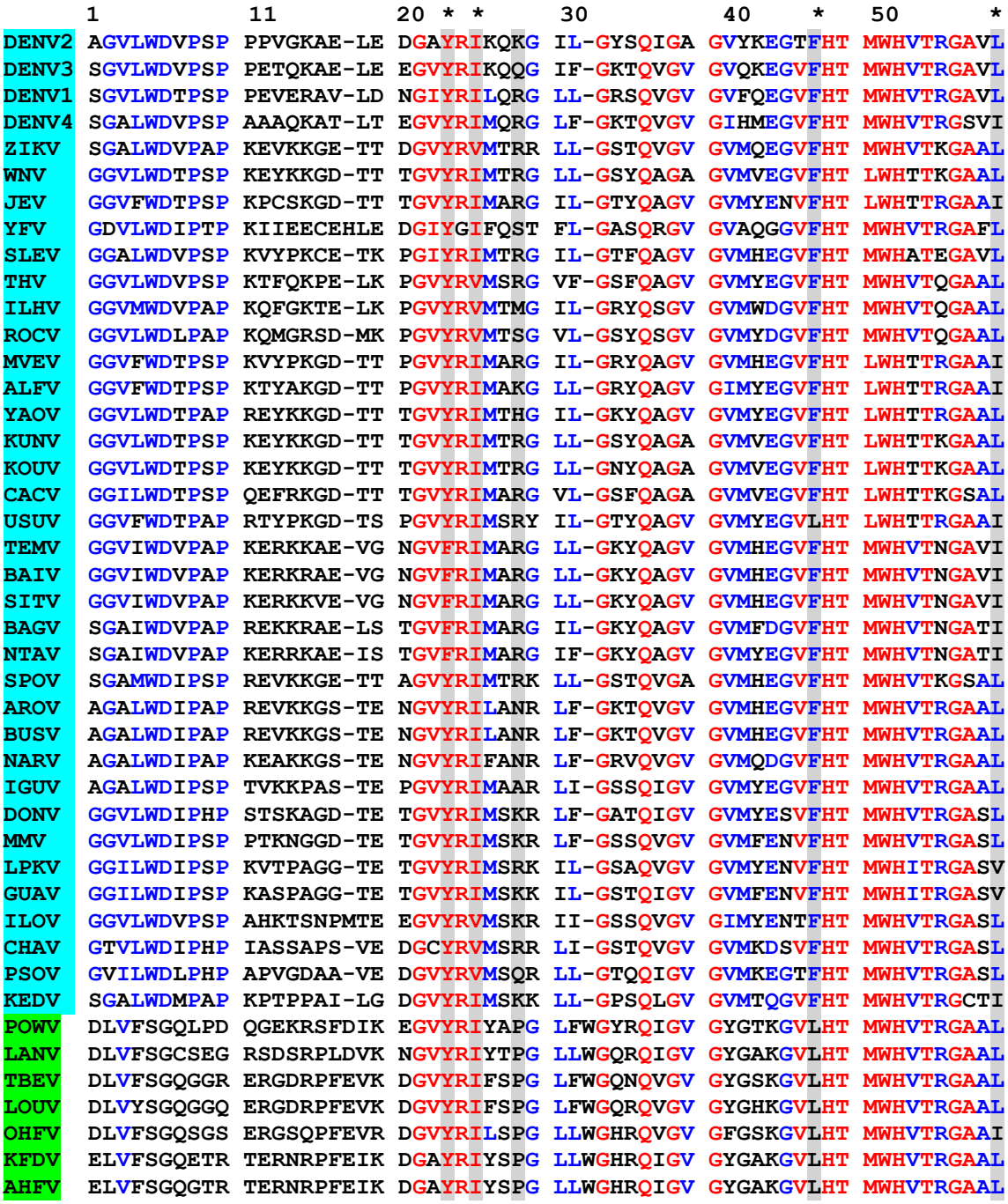
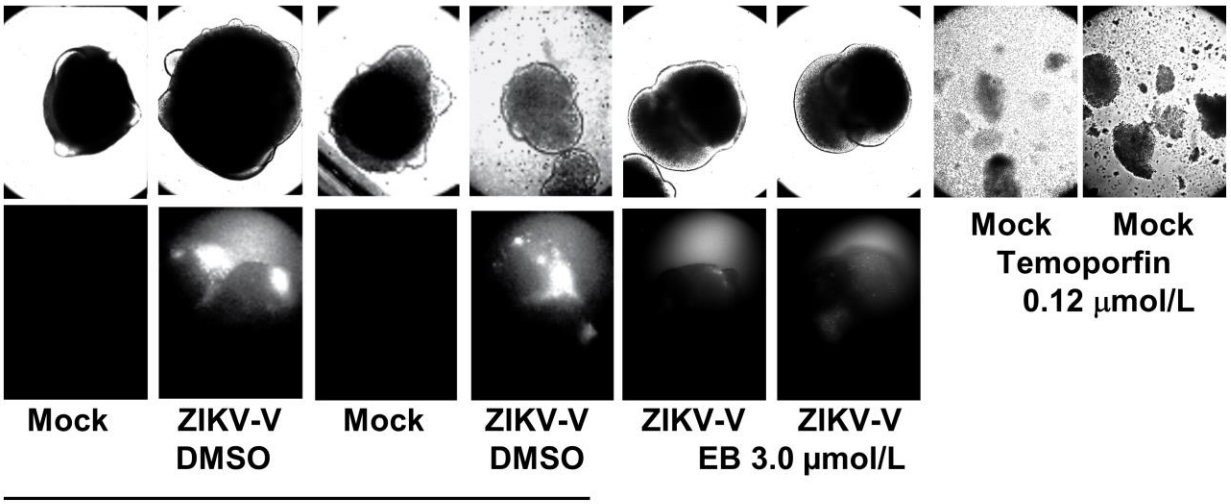


Figure S1 Alignment of the NS3 sequences at the EB-binding site of 44 representative flaviviruses. Residues are colored according to sequence conservation: Red, >90%; blue, >50%; black, <50%. The NS3 residues in contact erythrosin B are shaded, with essential residues for erythrosin B binding marked with *. Mosquito-borne viruses are shaded with cyan color; tick-borne viruses are shaded with green color. Virus Abbreviations: dengue virus (DENV); Zika virus (ZIKV); West Nile virus (WNV); Japanese encephalitis virus (JEV); yellow fever virus (YFV); St. Louis encephalitis virus (SLEV); T'Ho virus (THV); Ilheus virus (ILHV); Rocio virus (ROCV); Murray

Valley encephalitis virus (MVEV); Alfuy virus (ALFV); Yaounde virus (YAOV); Kunjin virus (KUNV); Koutango virus (KOUV); Cacipacore virus (CACV); Usutu virus (USUV); Tembusu virus (TEMV); Baiyangdian virus (BAIV); Sitiawan virus (SITV); Bagaza virus (BAGV); Ntaya virus (NTAV); Spondweni virus (SPOV); Aroa virus (AROV); Bussuquara virus (BUSV); Naranjal virus (NARV); Iguape virus (IGUV); Donggang virus (DONV); Marisma mosquito virus (MMV); Long Pine Key virus (LPKV); Guapiacu virus (GUAV); Ilomantsi virus (ILOV); Chaoyang virus (CHAV); Psorophora flavivirus (PSOV); Kedougou virus (KEDV); Powassan virus (POWV); Langat virus (LANV); Tick-borne encephalitis virus (TBEV); Louping ill virus (LOUV); Omsk hemorrhagic fever virus (OHFV); Kyasanur Forest disease virus (KFDV); Alkhumra hemorrhagic fever virus (AHFV).



Published previously, refs[35, 57]

Figure S2, ZIKV organoid infected with ZIKV-Venus (ZIKV-V)[35, 37]. The 3D organoids were infected with PBS (Mock), or ZIKV untreated (DMSO), or ZIKV treated with EB (3.0 μmol/L), or Mock treated with Temoporfin (0.12 μmol/L). Upper panel, bright field image of intact organoids. Lower panel, Venus fluorescence image (excitation 515 nm, emission 528 nm) of the intact 3D organoids.