

## Supporting Information for

### Original article

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

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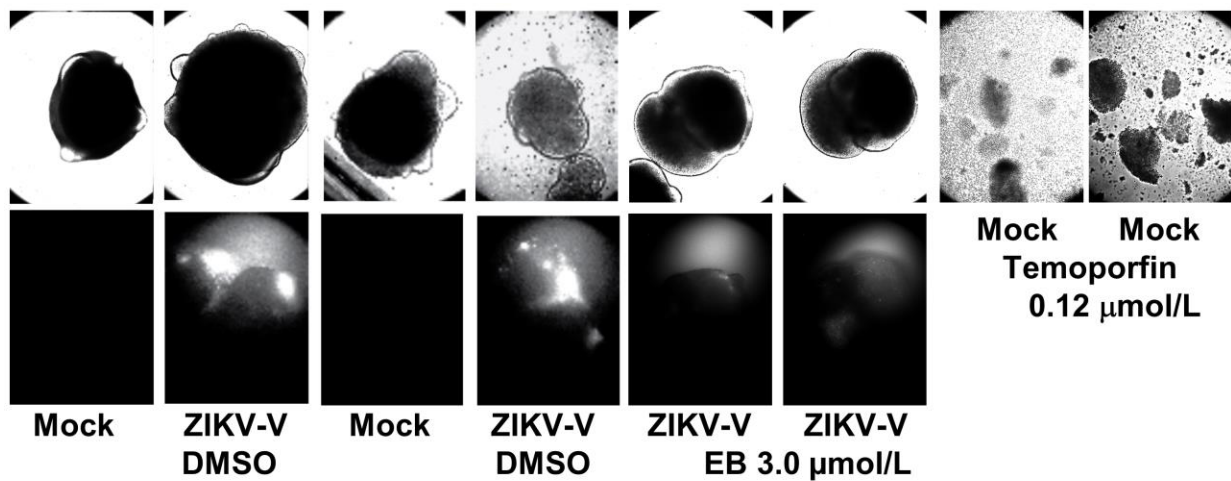
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	1	11	20 * *	30	40 *	50 *
DENV2	AGVLWDVPSP	PPVGKAE-LE	DGAYRIKQKG	IL-GYSQIGA	GVYKEGTFHT	MWHVTRGAVL
DENV3	SGVLWDVPSP	PETQKAE-LE	EGVYRIKQQG	IF-GKTQVGV	GVQKEGVFHT	MWHVTRGAVL
DENV1	SGVLWDTSP	PEVERAV-LD	NGIYRILQRG	LL-GRSQVGV	GVFQEGVFHT	MWHVTRGAVL
DENV4	SGALWDVPSP	AAAQKAT-LT	EGVYRIMQRG	LF-GKTQVGV	GIHMEGVFHT	MWHVTRGSVI
ZIKV	SGALWDVPAP	KEVKKGE-TT	DGVYRVMTRR	LL-GSTQVGV	GVMQEGVFHT	MWHVTKGAAL
WNV	GGVLWDTSP	KEYKKGD-TT	TGVYRIMTRG	LL-GSYQAGA	GVMVEGVFHT	LWHTTKGAAL
JEV	GGVFWDTPSP	KPCSKGD-TT	TGVYRIMARG	IL-GTYQAGV	GVMYENVFHT	LWHTTRGAAI
YFV	GDVLWDIPTP	KIIEECEHLE	DGIYGIFQST	FL-GASQRGV	GVAQGGVFHT	MWHVTRGAFL
SLEV	GGALWDVPSP	KVYPKCE-TK	PGIYRIMTRG	IL-GTFQAGV	GVMHEGVFHT	MWHATEGAVL
THV	GGVLWDVPSP	KTFQKPE-LK	PGVYRVMSRG	VF-GSFQAGV	GVMYEGVFHT	MWHVTQGAAL
ILHV	GGVMWDVPAP	KQFGKTE-LK	PGVYRVMTMG	IL-GRYQSGV	GVMWDGVFHT	MWHVTQGAAL
ROCV	GGVLWDLAP	KQMGSRD-MK	PGVYRVMTSG	VL-GSYQSGV	GVMYDGVFHT	MWHVTQGAAL
MVEV	GGVFWDTPSP	KVYPKGD-TT	PGVYRIMARG	IL-GRYQAGV	GVMHEGVFHT	LWHTTRGAAI
ALFV	GGVFWDTPSP	KTYAKGD-TT	PGVYRIMAKG	LL-GRYQAGV	GIMYEGVFHT	LWHTTRGAAI
YAOV	GGVLWDTAP	REYKKGD-TT	TGVYRIMTHG	IL-GKYQAGV	GVMYEGVFHT	LWHTTRGAAL
KUNV	GGVLWDTSP	KEYKKGD-TT	TGVYRIMTRG	LL-GSYQAGA	GVMVEGVFHT	LWHTTKGAAL
KOUV	GGVLWDTSP	KEYKKGD-TT	TGVYRIMTRG	LL-GNYQAGA	GVMVEGVFHT	LWHTTKGAAL
CACV	GGILWDTSP	QEFRKGD-TT	TGVYRIMARG	VL-GSFQAGA	GVMVEGVFHT	LWHTTKGSAL
USUV	GGVFWDTPAP	RTYPKGD-TS	PGVYRIMSRY	IL-GTYQAGV	GVMYEGVLHT	LWHTTRGAAI
TEMV	GGVIWDVPAP	KERKKAEE-VG	NGVFRIMARG	LL-GKYQAGV	GVMHEGVFHT	MWHVTNGAVI
BAIV	GGVIWDVPAP	KERKRAE-VG	NGVFRIMARG	LL-GKYQAGV	GVMHEGVFHT	MWHVTNGAVI
SITV	GGVIWDVPAP	KERKKVE-VG	NGVFRIMARG	LL-GKYQAGV	GVMHEGVFHT	MWHVTNGAVI
BAGV	SGAIWDVPAP	KERKRAE-LS	TGVFRIMARG	IL-GKYQAGV	GVMFDGVFHT	MWHVTNGATI
NTAV	SGAIWDVPAP	KERRKAE-IS	TGVFRIMARG	IF-GKYQAGV	GVMYEGVFHT	MWHVTNGATI
SPOV	SGAMWDIPSP	REVKKGE-TT	AGVYRIMTRK	LL-GSTQVGA	GVMHEGVFHT	MWHVTKGSAL
AROV	AGALWDIPAP	REVKKGS-TE	NGVYRILANR	LF-GKTQVGV	GVMHEGVFHT	MWHVTRGAAL
BUSV	AGALWDIPAP	REVKKGS-TE	NGVYRILANR	LF-GKTQVGV	GVMHEGVFHT	MWHVTRGAAL
NARV	AGALWDIPAP	KEAKKGS-TE	NGVYRIFANR	LF-GRVQVGV	GVMQDGVFHT	MWHVTRGAAL
IGUV	AGALWDIPSP	TVKKPAS-TE	PGVYRIMAAR	LI-GSSQIGV	GVMYEGVFHT	MWHVTRGAAL
DONV	GGVLWDIPHP	STSKAGD-TE	TGVYRIMSKR	LF-GATQIGV	GVMYESVFHT	MWHVTRGASL
MMV	GGVLWDIPSP	PTKNGGD-TE	TGVYRIMSKR	LF-GSSQVGV	GVMFENVFHT	MWHVTRGASL
LPKV	GGILWDIPSP	KVTPAGG-TE	TGVYRIMSRK	IL-GSAQVGV	GVMYENVFHT	MWHITRGASV
GUAV	GGILWDIPSP	KASPAGG-TE	TGVYRIMSKK	IL-GSTQIGV	GVMFENVFHT	MWHITRGASV
ILOV	GGVLWDVPSP	AHKTSNPMTE	EGVYRVMSKR	II-GSSQVGV	GIMYENTFHT	MWHVTRGASL
CHAV	GTVLWDIPHP	IASSAPS-VE	DGCYRVMSRR	LI-GSTQVGV	GVMKDSVFHT	MWHVTRGASL
PSOV	GVILWDLPHP	APVGDAE-VE	DGVYRVMSQR	LL-GTQQIGV	GVMKEGTFHT	MWHVTRGASL
KEDV	SGALWDMPP	KPTPPAI-LG	DGVYRIMSKK	LL-GPSQLGV	GVMTQGVFHT	MWHVTRGCTI
POWV	DLVFSGQLPD	QGEKRSFDIK	EGVYRIYAPG	LFWGYRQIGV	GYGTKGVLHT	MWHVTRGAAL
LANV	DLVFSGCSEG	RSDSRPLDVK	NGVYRIYTPG	LLWGQRQIGV	GYGAKGVLHT	MWHVTRGAAL
TBEV	DLVFSGQGG	ERGDRPFVVK	DGVYRIFSPG	LFWGQNQVGV	GYGSKGVLHT	MWHVTRGAAL
LOUV	DLVYSGQGG	ERGDRPFVVK	DGVYRIFSPG	LFWGQRQVGV	GYGHKGVLHT	MWHVTRGAAL
OHFV	DLVFSGQSGS	ERGSQPFVVR	DGVYRILSPG	LLWGHQRQVGV	GFGSKGVLHT	MWHVTRGAAI
KFDV	ELVFSGQETR	TERNRPFVVK	DGAYRIYSPG	LLWGHQRQIGV	GYGAKGVLHT	MWHVTRGAAL
AHFV	ELVFSGQGTR	TERNRPFVVK	DGAYRIYSPG	LLWGHQRQIGV	GYGAKGVLHT	MWHVTRGAAL

**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<sup>35, 57</sup>

**Figure S2**, ZIKV organoid infected with ZIKV-Venus (ZIKV-V)<sup>35, 37</sup>. The 3D organoids were infected with PBS (Mock), or ZIKV untreated (DMSO), or ZIKV treated with EB (3.0  $\mu\text{mol/L}$ ), or Mock treated with Temoporfin (0.12  $\mu\text{mol/L}$ ). Upper panel, bright field image of intact organoids. Lower panel, Venus fluorescence image (excitation 515 nm, emission 528 nm) of the intact 3D organoids.