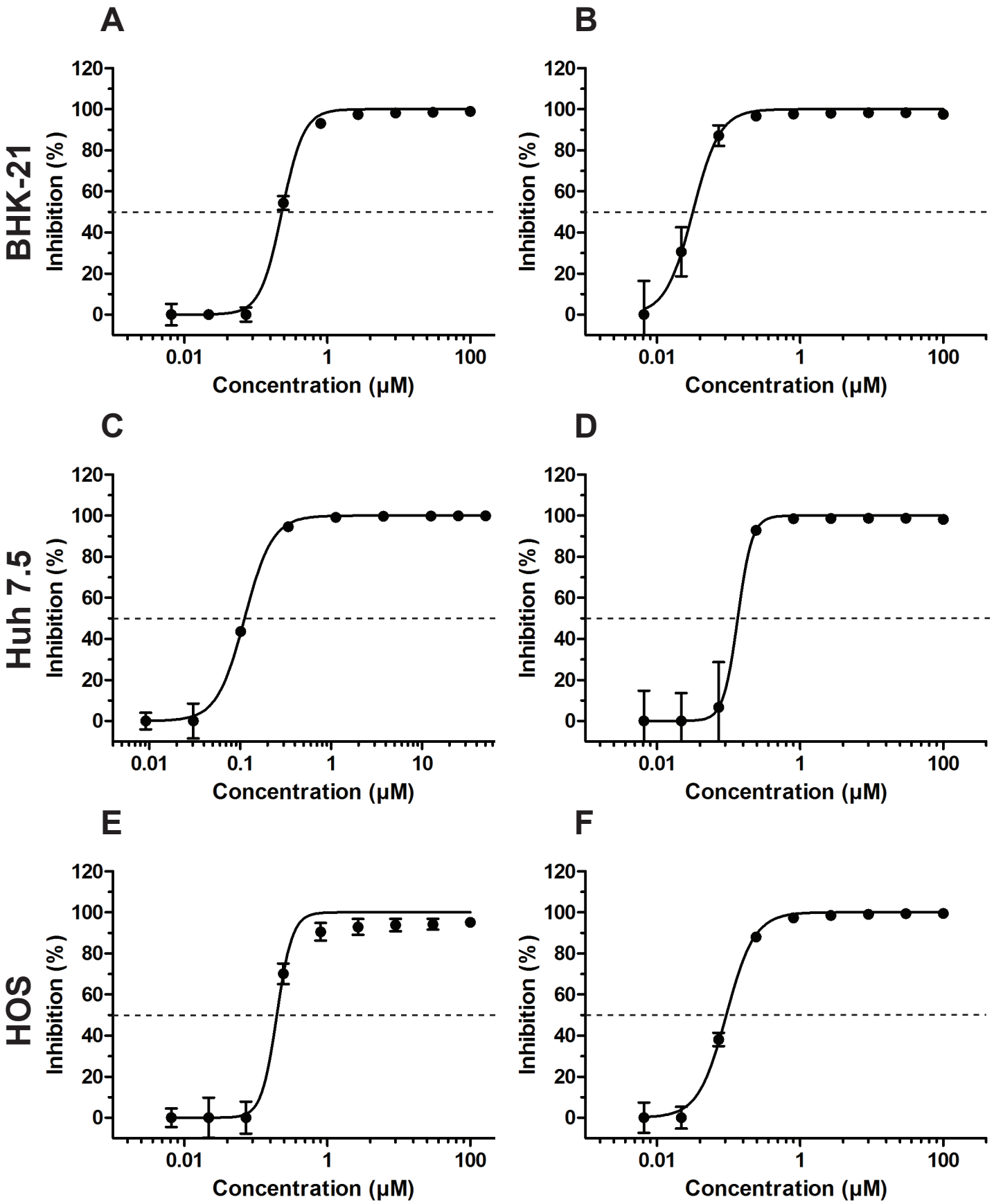

Figure S1. Obatoclax inhibits alphaviruses at low micromolar concentrations. Dose response assay to evaluate the antiviral activity of OLX against SFV and CHIKV in different cell lines. **(A)** and **(B)** BHK-21 cells infected at MOI 0.01 with SFV **(A)** and CHIKV **(B)** for 16 h. **(C)** and **(D)** Huh 7.5 cells infected at MOI 0.1 with SFV **(C)** for 12 h and CHIKV **(D)** for 18 h. **(E)** and **(F)** HOS cells infected at MOI 1 with SFV **(E)** for 16 h and CHIKV **(F)** for 20 h. Infections were performed in the presence of obatoclax at concentrations ranging from 0.007 µM – 100 µM. Percent inhibition values were calculated based on luciferase signals from infected cells treated with 0.1% DMSO. Half maximal (EC_{50}) threshold is marked with a dotted line. Assays were performed in triplicate wells. Data are presented as means ± SEM (n = 3 for BHK-21, n = 2 for Huh 7.5, n = 2 for HOS).

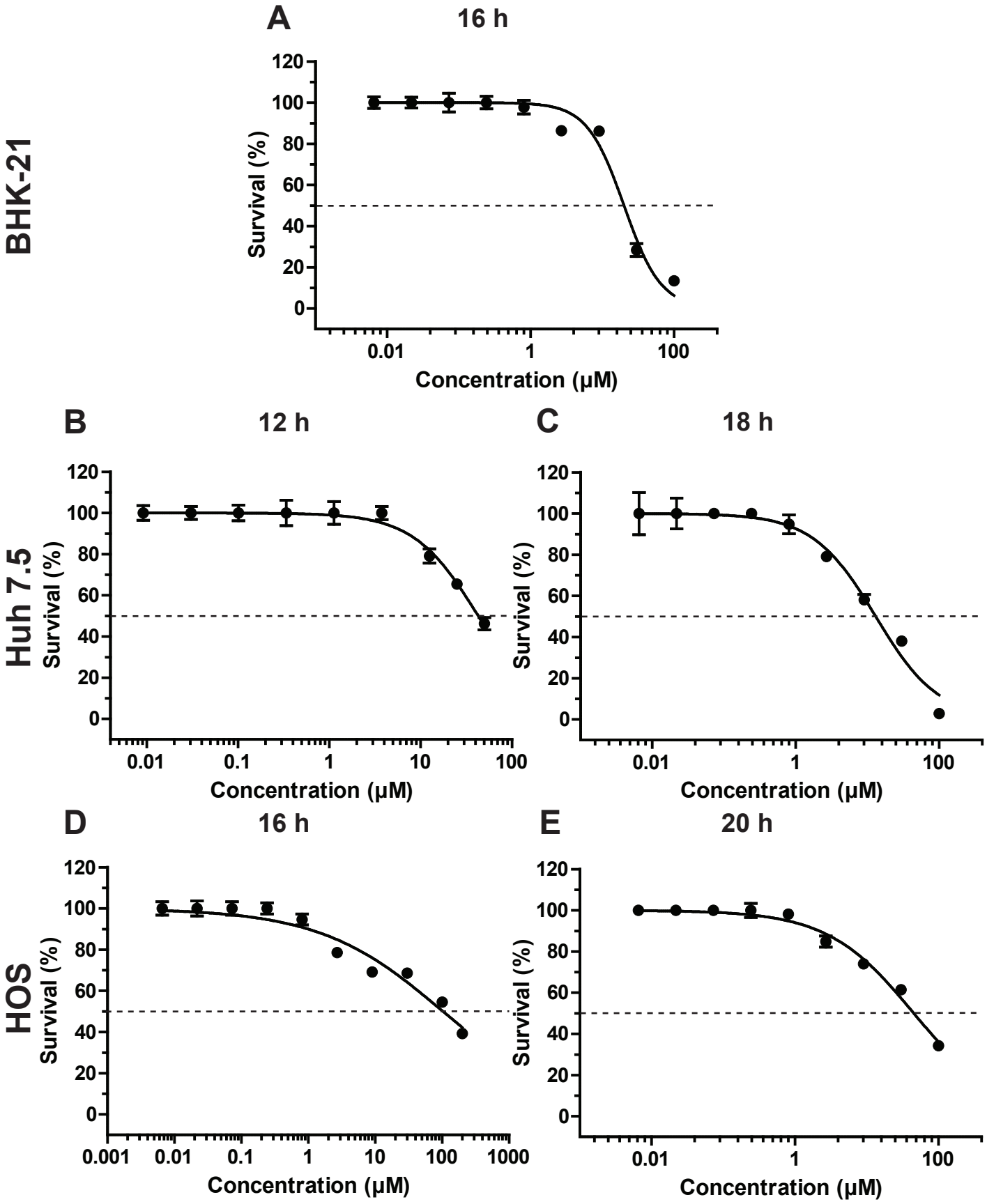

Figure S2. Cytotoxicity assays with obatoclax. (A – E) Cell-survival dose-response assay using the CTG assay to assess the cytotoxicity of obatoclax in different cell lines. Obatoclax treatment at concentrations ranging from 0.007 µM – 100 µM was given to BHK-21 cells **(A)** for 16 h, Huh 7.5 cells for 12 h **(B)** or 18 h **(C)**, HOS cells for 16 h **(D)** or 20 h **(E)**. Percent survival values were calculated based on luciferase signals from untreated mock cells. Half maximal (CC_{50}) threshold is marked with a dotted line. Assays were performed in triplicate wells. Data are presented as means ± SEM (n = 3 for BHK-21, n = 2 for Huh 7.5, n = 2 for HOS).

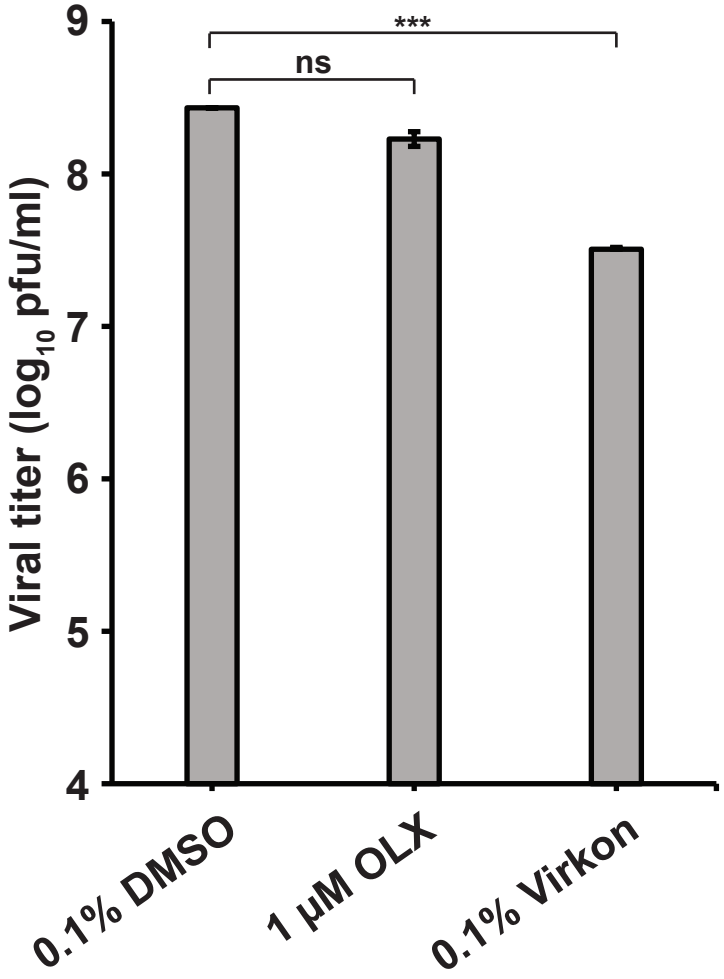

Figure S3. Assessing the virucidal activity of obatoclax. Undiluted stock of wild type CHIKV was treated with 0.5 µM OLX at 37°C for 30 min. The treated stock was then titrated on BHK-21 cells using plaque assay. 0.1% DMSO and 0.1% Virkon were used as negative and positive controls respectively. Data from two independent experiments. Error bars represent standard error of means. Statistical significance was determined using one-way ANOVA test. (***; $p < 0.001$).

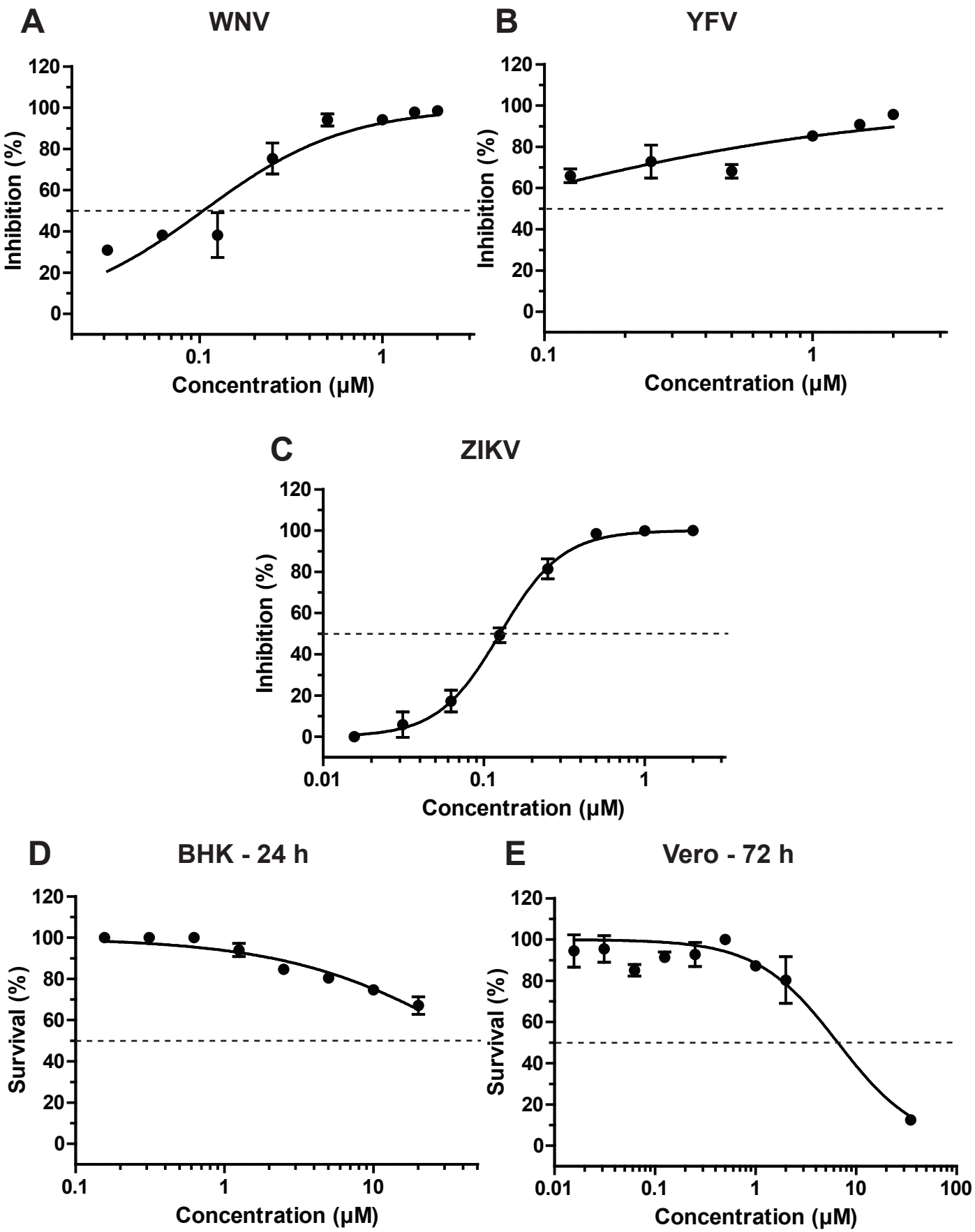

Figure S4. Antiviral activity of obatoclax against flaviviruses. (A) and **(B)** BHK-21 cells were infected with WNV (isolate NY 2000-crow3356) **(A)** or YFV (17D strain) **(B)** at MOI 0.01. Infection was performed in Glasgow Minimum Essential Medium (GMEM) containing 1% FBS with OLX at concentrations ranging from 2 μM – 0.016 μM (**A**; WNV) or from 2 μM – 0.125 μM (**B**; YFV). Following 1 h adsorption, medium was replaced with full cell culture medium (GMEM, 5% FBS, 10% tryptose, 2% HEPES, 1% penicillin-streptomycin, 1% L-glutamine) containing the same concentration of inhibitors. At 24 h p.i., cell culture supernatants were collected and subjected to plaque assay titration in duplicate. Percent inhibition values were calculated based on titers from samples containing 0.1% DMSO. Data are presented as means ± SEM and are representative of two independent experiments. **(C)** Vero E6 cells were infected ZIKV-UbiNanoLuc at MOI 0.01. Infection was performed in IMDM containing 2% FBS with OLX at concentrations ranging from 2 μM – 0.016 μM. At 72 h p.i., cells were lysed and luciferase values were measured. Percent inhibition values were calculated based on luciferase values from infected samples containing 0.1% DMSO. Half maximal (EC_{50}) threshold is marked with a dotted line. **(D)** and **(E)** Cell survival dose-response assays done in parallel to evaluate the cytotoxicity of OLX. Obatoclax treatment at concentrations ranging from 0.16 μM – 20 μM was given to BHK-21 cells **(D)** and treatment from 0.16 μM – 35 μM to Vero E6 cells **(E)** under the same media conditions used for the respective infection assays. Cytotoxicity was measured using an MTT assay and percent survival values were calculated based on absorbance values from untreated mock cells. Half maximal (CC_{50}) threshold is marked with a dotted line. Assays were performed in triplicate wells. Data are presented as means ± SEM (n = 2).

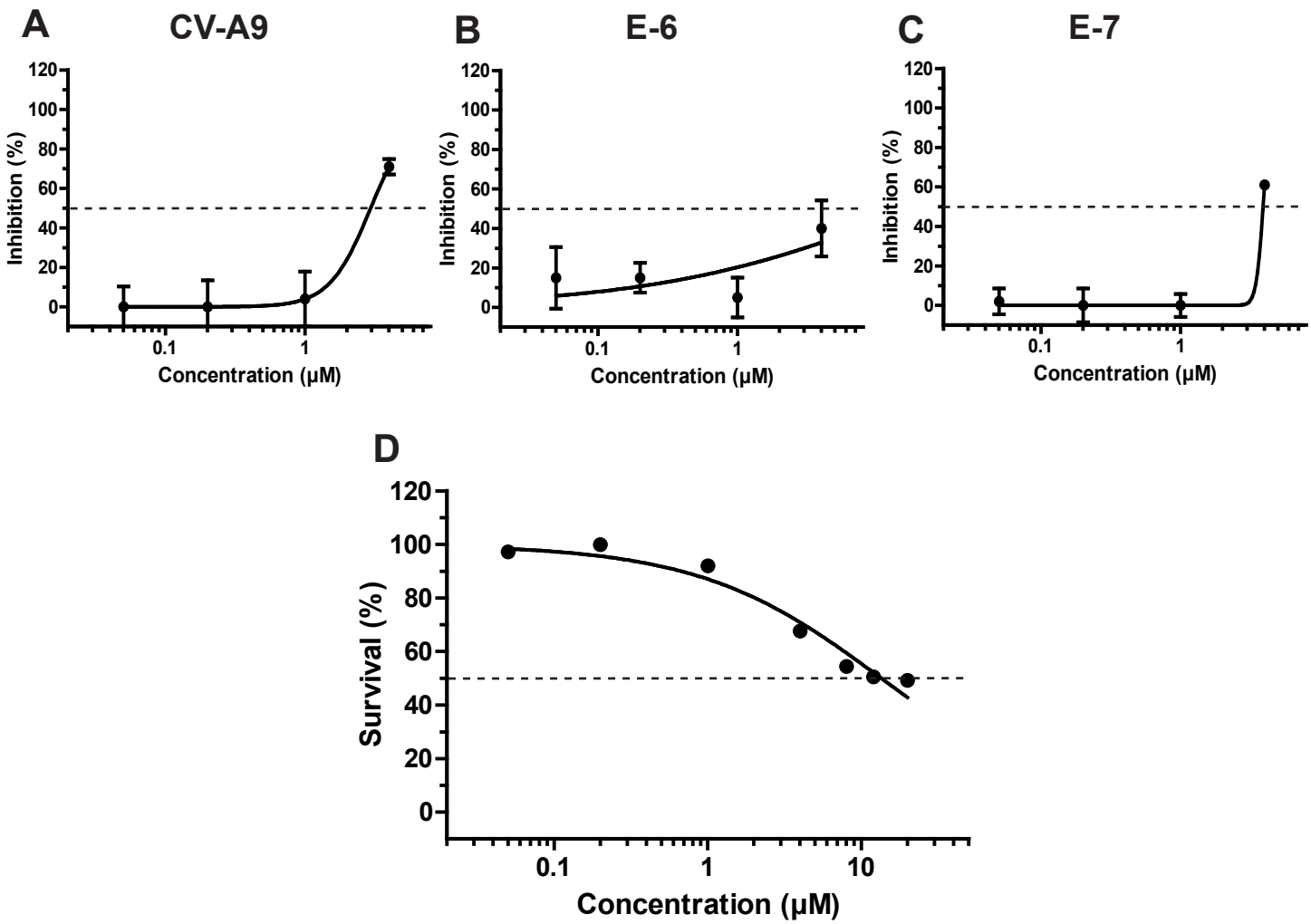

Figure S5. The effect of obatoclax on pH-independent picornaviruses. Dose response assay to evaluate the antiviral activity of OLX against different pH-independent picornaviruses. A549 cells were infected with CV-A9 **(A)**, E-6 **(B)** and E-7 **(C)** at 20% efficiency of infection for 1 h on ice, followed by addition of medium containing OLX at concentrations ranging from 0.05 μM – 4 μM. At 6 h p.i., cells were processed for indirect immunofluorescence using virus-specific antibodies and percent infection values were calculated compared to untreated controls. Half maximal (EC_{50}) threshold is marked with a dotted line. Assays were performed in five replicate wells. Data are presented as means ± SEM (n = 2). **(D)** Cell survival dose-response assay done in parallel to evaluate the cytotoxicity of OLX. Obatoclax treatment at concentrations ranging from 0.05 μM – 20 μM was given to A549 cells under the same media conditions used for the infection assay. Cytotoxicity was measured using CCK-8 kit and percent survival values were calculated based on absorbance values from untreated mock cells. Half maximal (CC_{50}) threshold is marked with a dotted line. Assays were performed in triplicate wells. Data are presented as means ± SEM (n = 2).