Broad-range inhibition of enterovirus replication by OSW-1, a natural compound targeting OSBP

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Abstract

Enteroviruses, e.g., polio-, coxsackie- and rhinoviruses, constitute a large genus within the *Picornaviridae* family of positive-strand RNA viruses and include many important pathogens linked to a variety of acute and chronic diseases. Despite their huge medical and economic impact, no approved antiviral therapy is yet available. Recently, the oxysterol-binding protein (OSBP) was implicated as a host factor for enterovirus replication. Here, we investigated the antiviral activity of the natural compound OSW-1, a ligand of OSBP that is under investigation as an anti-cancer drug. OSW-1 potently inhibited the replication of all enteroviruses tested, with IC_{50} values in the low nanomolar range, acted at the genome replication stage and was effective in all tested cell types of three different species. Importantly, OSBP overexpression rescued viral replication, demonstrating that the antiviral effect of OSW-1 is due to targeting OSBP. Together, we here report the anti-enterovirus activity of the natural anti-cancer compound OSW-1.

Keywords: enterovirus; replication; oxysterol-binding protein; coxsackievirus; antiviral.

Abbreviations

CC_{50}, 50% cytotoxic concentration; CVB3, coxsackievirus B3; ERAV, equine rhinitis A virus; EV, enterovirus; FLuc, Firefly luciferase; HPeV1, human parechovirus 1; HRV, human rhinovirus; IC_{50}, 50% inhibitory concentration; MHV, murine hepatitis virus; OSBP, oxysterol-binding protein; PI4KIIIβ, phosphatidylinositol 4-kinase type III beta; PI4P, phosphatidylinositol 4-phosphate; RLuc, *Renilla* luciferase; VSV, vesicular stomatitis virus; wt, wild type

Enteroviruses form a large genus belonging to the *Picornaviridae* family of positive-strand RNA viruses [(+)RNA] and include important human pathogens. Enteroviral infections have been implicated in a number of acute and chronic diseases, ranging from poliomyelitis (poliovirus), meningoencephalitis and myocarditis (coxsackieviruses and echoviruses), and common cold to asthma exacerbation and chronic obstructive pulmonary disease (rhinoviruses). Poliovirus is the only member of the genus for which an efficient vaccine is available and no antiviral therapy is currently approved for treating enteroviral infections.

Efficient genome replication of enteroviruses is associated with virus-induced remodelling of intracellular membranes and alterations in lipid homeostasis (Belov and van Kuppeveld, 2012). During this process, enteroviruses hijack a number of host cell factors, including GBF1 and PI4KIIIβ (Belov et al., 2007; Hsu et al., 2010; Lanke et al., 2009). Replication organelles of enteroviruses are enriched in PI4P lipids produced by PI4KIIIβ (Hsu et al., 2010). Recently, oxysterol-binding protein (OSBP), a PI4P-binding protein that shuttles cholesterol between membrane compartments, was implicated as another host factor for enterovirus replication (Arita, 2014; Arita et al., 2013; Roulin et al., 2014; Wang et al., 2014).

OSW-1 is a natural compound extracted from the bulbs of the plant *Ornithogalum saundersiae* that has been studied mainly for its anti-cancer activity. Burgett et al. (2011) identified OSBP as a high-affinity target of OSW-1 using affinity chromatography and demonstrated that OSW-1 exerts its anti-cancer activity via OSBP.

In this study, we investigated the antiviral activity of OSW-1. We assessed the effect of OSW-1 on a single-round of infection of HeLa or Buffalo Green Monkey (BGM) kidney cells by viruses from different species in the *Enterovirus* genus, i.e., enterovirus 71 (EV71, enterovirus A species), coxsackievirus A21 (CVA21, enterovirus C species), human rhinovirus 2 (HRV2, rhinovirus A species) and human rhinovirus 14 (HRV-14, rhinovirus B species). All enteroviruses tested were sensitive to OSW-1, with IC_{50} values ranging between 2.4 and 9.4 nM (Fig. 1A-E). Cell viability assays performed in parallel revealed no cytotoxicity (CC_{50} >

100nM, Fig. 1B,F) within the time frame of these infections (i.e., 10 h). Likewise, coxsackievirus B3 (CVB3, enterovirus B species) expressing *Renilla* luciferase (RLuc-CVB3) was also potently inhibited by OSW-1 (Fig. 1I and 2). We also tested the effects of OSW-1 on replication of some other picornaviruses. Equine rhinitis A virus (ERAV), a member of the *Aphthovirus* genus, was slightly inhibited at higher OSW-1 concentrations, but these concentrations were also found to affect cell viability upon 24 h incubation, i.e., the time point after which virus replication was assessed (Fig. 1G). Human parechovirus 1 (HPeV1), a member of the *Parechovirus* genus, was not inhibited (data not shown). These data indicate that not all picornaviruses require OSBP for replication.

Recently, OSW-1 was shown to also inhibit the replication of another (+)RNA virus, i.e., hepatitis C virus, a member of the *Flaviviridae* family, which requires OSBP as a host factor for replication as well (Wang et al., 2014). We wanted to study whether OSW-1 could also inhibit viruses from other large families. Therefore, we tested the effect of OSW-1 on the replication of two other viruses, murine hepatitis virus (MHV), a (+)RNA virus from the *Coronaviridae* family, and vesicular stomatitis virus (VSV), a (–)RNA virus from the *Rhabdoviridae* family. Replication of the firefly luciferase-expressing reporter viruses MHV-EFLM (de Haan et al., 2003) or VSVΔG/FLuc-G* (Burkard et al., 2014) was not inhibited by OSW-1, while RLuc-CVB3 replication was significantly inhibited (Fig. 1I).

Next, we tested whether the antiviral activity of OSW-1 is cell-type dependent. Cell lines from three species, i.e., human (HeLa R19 and HAP1 cells), monkey (BGM and Vero cells) and mouse (mouse embryonic fibroblasts, MEF) were infected with RLuc-CVB3 and the antiviral activity of OSW-1 was assessed. OSW-1 inhibited virus replication in all cell types tested (Fig. 2) without cytotoxic effects, although IC_{50} values varied somewhat between cells (e.g., HAP1 cells, 0.2 nM; BGM cells, 2.4 nM). These findings demonstrate that OSW-1 can inhibit virus replication in different cell types from different species.

To study which step of the replication cycle is inhibited by OSW-1, we first performed a time-of-addition experiment in which we added OSW-1 at different time points after infection. OSW-1 treatment had a similar profile as the established replication inhibitor guanidine HCl (Barton and Flanegan, 1997) and strongly inhibited replication when added up to 3 h after infection, indicating that not entry but rather a step during genome replication was inhibited by OSW-1 (Fig. 3A). To confirm that OSW-1 inhibits the replication stage, we transfected a CVB3 subgenomic replicon containing firefly luciferase in place of the capsid-coding region [FLuc-CVB3 (Wessels et al., 2005)]. We observed that OSW-1 had a similar inhibitory activity against both the virus and the replicon (Fig. 3B), which confirms that OSW-1 targets the genome replication stage.

In poliovirus, a mutation in 3A (A70T) provides cross-resistance to PI4KIIIβ inhibitors and two presumed inhibitors of OSBP, AN-12-H5 and T-00127-HEV2 (Arita et al., 2013; Arita et al., 2010). We wanted to test whether the mutation H57Y in the 3A protein of CVB3, which provides resistance to PI4KIIIβ inhibitors such as BF73735 (van der Schaar et al., 2013), also provided resistance to OSW-1. Using RLuc-CVB3 wt and 3A[H57Y] reporter viruses, we observed that the mutation also protected to a similar degree to OSW-1 (Fig. 4A). Cross-resistance of the 3A[H57Y] mutant to PI4KIIIβ inhibitors and OSW-1 suggests that PI4KIIIβ and OSBP act in the same pathway and is in line with previous reports that OSBP is recruited by PI4KIIIβ-generated PI4P (Arita, 2014; Arita et al., 2013; Roulin et al., 2014).

To further confirm that OSW-1 exerts its antiviral activity via OSBP, we tested whether OSBP overexpression could restore virus replication in the presence of OSW-1. Using this assay, we previously demonstrated that overexpression of (a drug-resistant mutant of) PI4KIIIβ could rescue replication from the inhibitory effect of PI4KIIIβ-inhibitors (van der Schaar et al., 2013; van der Schaar et al., 2012). Overexpression of OSBP restored CVB3 replication in the presence of OSW-1 but not BF738735 (Fig. 4B), whereas overexpression of PI4KIIIβ protected

replication only against BF738735 but not OSW-1 (Fig. 4B). These data support our conclusion that OSW-1 specifically inhibits enterovirus replication through OSBP.

A number of other compounds were recently suggested to inhibit enterovirus replication by targeting OSBP. These include AN-12-H5 (Arita et al., 2010) and T-00127-HEV2 (Arita et al., 2013), which were dubbed "minor enviroxime-like compounds" because viruses that were selected for resistance against these compounds contain the same mutation that was previously shown to provide resistance to the PI4KIIIβ inhibitor enviroxime. AN-12-H5 and T-00127-HEV2 are thought to target OSBP because OSBP knockdown sensitized poliovirus to these compounds and because they caused a redistribution of OSBP (Arita et al., 2013). However, these compounds have not been shown to bind OSBP and it remains to be shown whether they are *bona fide* OSBP ligands. The promiscuous OSBP ligand 25-hydroxycholesterol also inhibits enterovirus replication, albeit weakly (Arita et al., 2013; Roulin et al., 2014); (our unpublished observations), but 25-hydroxycholesterol targets many other proteins as well, including other members of the family of OSBP-related proteins (ORPs) and proteins involved in cholesterol synthesis.

In conclusion, we here report that OSW-1 is a broad-range inhibitor of enterovirus genome replication that targets the host factor OSBP. This is the first report of a potent, known OSBP ligand, isolated from a natural source, that inhibits enterovirus replication.

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Figure legends.

Figure 1. OSW-1 is a broad-range inhibitor of enteroviruses

(A-B) BGM cells were infected EV71 at an MOI of 1 and treated with the indicated concentrations of OSW-1. Virus titers were determined at 10 h post infection (p.i.) by endpoint titration according to the method of Reed and Muench and expressed as 50% cell culture infective doses ($CCID_{50}$) (A). A cell viability assay was performed in parallel using MTS (Promega). **(C-F)** HeLa R19 cells were infected with CVA-21 **(C),** HRV-2 **(D)** or HRV-14 **(E)** at an MOI of 1, treated with OSW-1 and virus titers were determined at 10 h p.i. A cell viability assay was performed in parallel **(F)**. **(G)** HeLa R19 cells were infected with ERAV at an MOI of 1 and treated with OSW-1, virus titers were determined at 24h p.i. and a cell viability was performed in parallel **(H)**. **(J)** HeLa-mCC1a cells (Burkard et al., 2014) were infected with RLuc-CVB3, or the MHV-EFLM or VSVΔG/FLuc-G* at an MOI of 1, treated with DMSO, 10 nM OSW-1 or 5 μg/mL BFA as a positive control, cells were lysed at 6 h p.i and luciferase levels were determined using the (*Renilla*) Luciferase Assay System kits (Promega). Experiments were performed in triplicate and mean values ± SEM are shown. Statistical significance between treatments and controls was assessed by unpaired one-tailed Student's t test (with Welch's correction applied where necessary); * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure 2. The inhibitory effect of OSW-1 is not cell-type dependent

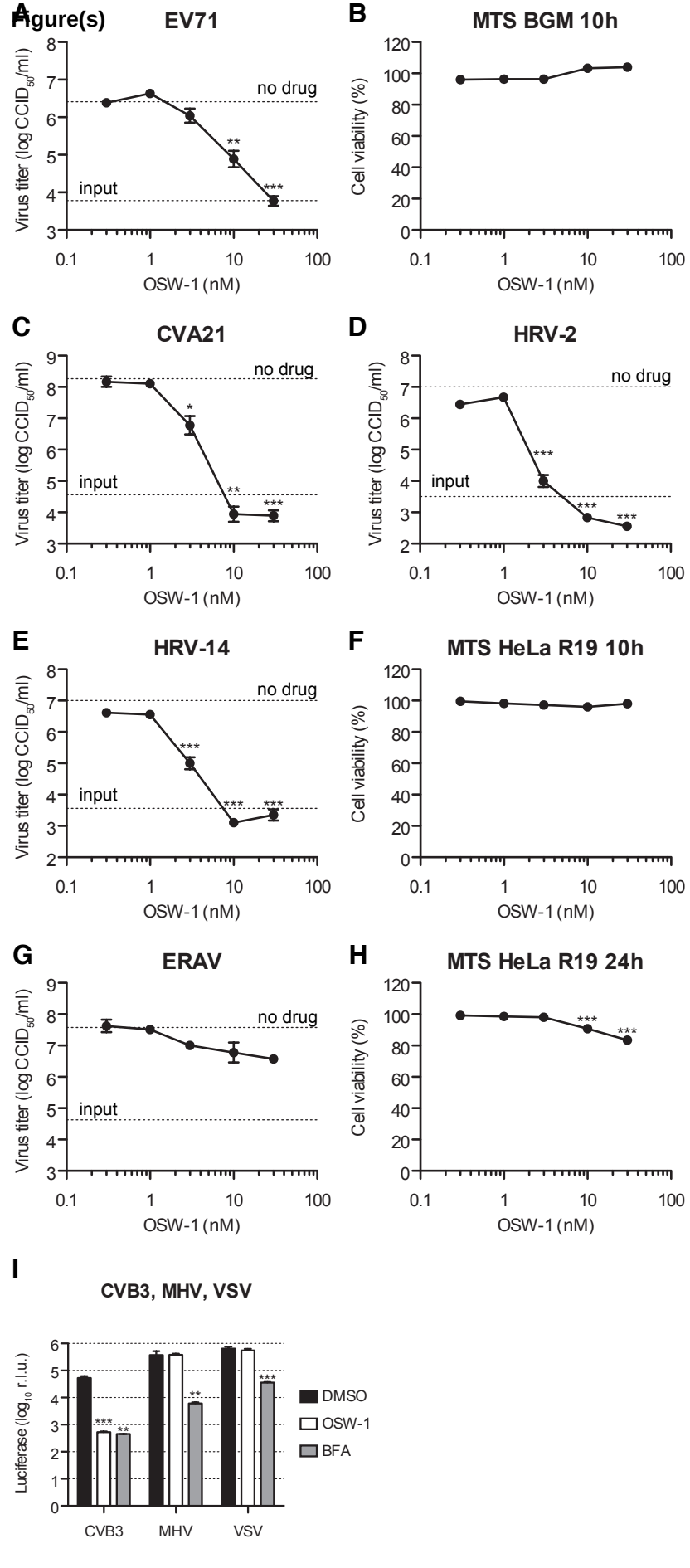
HeLa R19 **(A),** HAP1 **(B)**, BGM **(C),** Vero **(D)** or MEF **(E)** cells were infected with RLuc-CVB3 at an MOI of 0.1, treated with OSW-1 and luciferase levels at 7 h p.i. were determined. A cell viability assay was performed in parallel for each cell type. Experiments were performed and analyzed as in Fig. 1.

Figure 3. OSW-1 targets RNA genome replication

(A) HeLa R19 cells were infected with RLuc-CVB3 at an MOI of 0.1, 10nM OSW-1 or 2mM guanidine HCl (Gua) as a positive control was added at the indicated time point, and luciferase levels at 7 h p.i. were determined. **(B)** HeLa R19 cells were transfected with 5 ng FLuc-CVB3 replicon RNA, treated with 10 nM OSW-1, and luciferase levels at 7 h p.i. were determined. Experiments were performed in triplicate and analyzed as in Fig. 1.

Figure 4. OSW-1 inhibits enterovirus replication by targeting OSBP

(A) HeLa R19 cells were infected with either RLuc-CVB3 or RLuc-CVB3-3A[H57Y] at an MOI of 0.1, treated with DMSO, 3nM OSW-1, 1μM BF738735 (BF) or 2 mM Gua, and luciferase levels at 7 h p.i. were determined. **(B)** HeLa R19 cells were transfected for 24 h with constructs encoding OSBP, GalT-EGFP (negative control) or PI4KIIIβ[Y583M] using Fugene (Promega) , infected with RLuc-CVB3 at MOI 0.1, and treated with 3 nM OSW-1, 1 μM BF738735 (BF) or DMSO, and luciferase levels at 7 hr p.i. were determined. Experiments were performed in triplicate and analyzed as in Fig. 1.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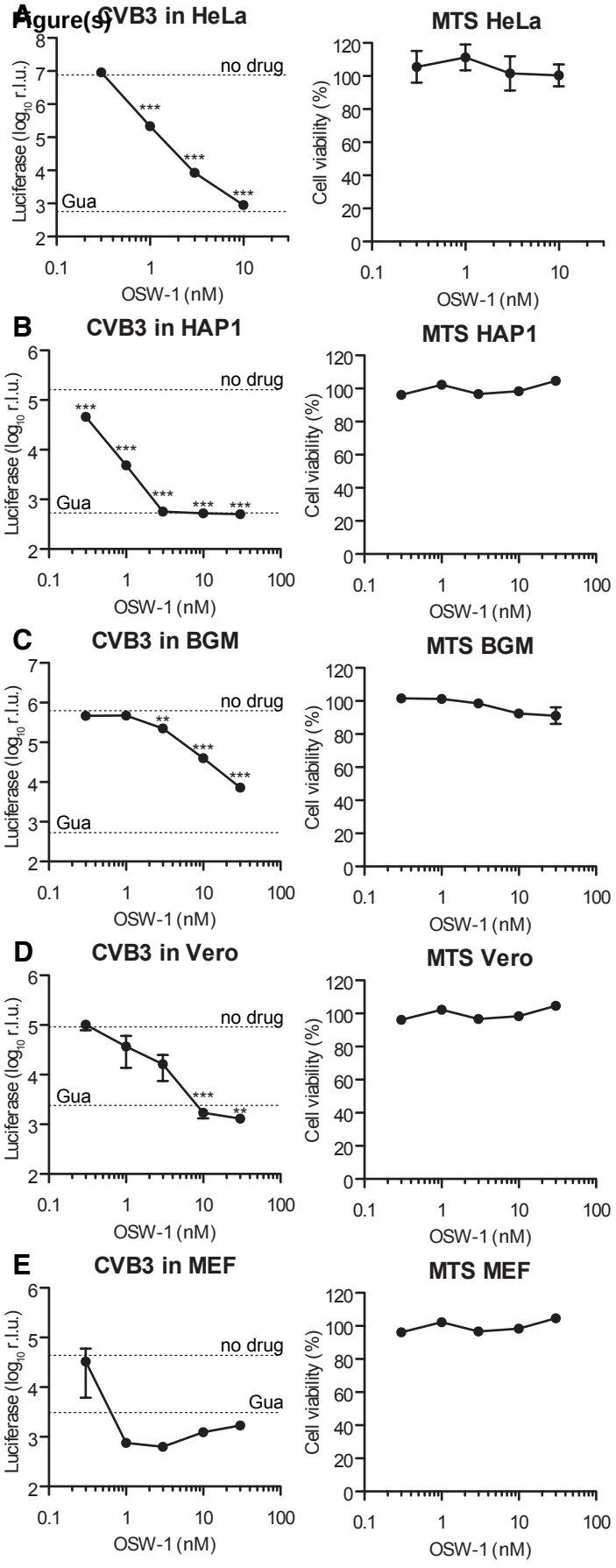
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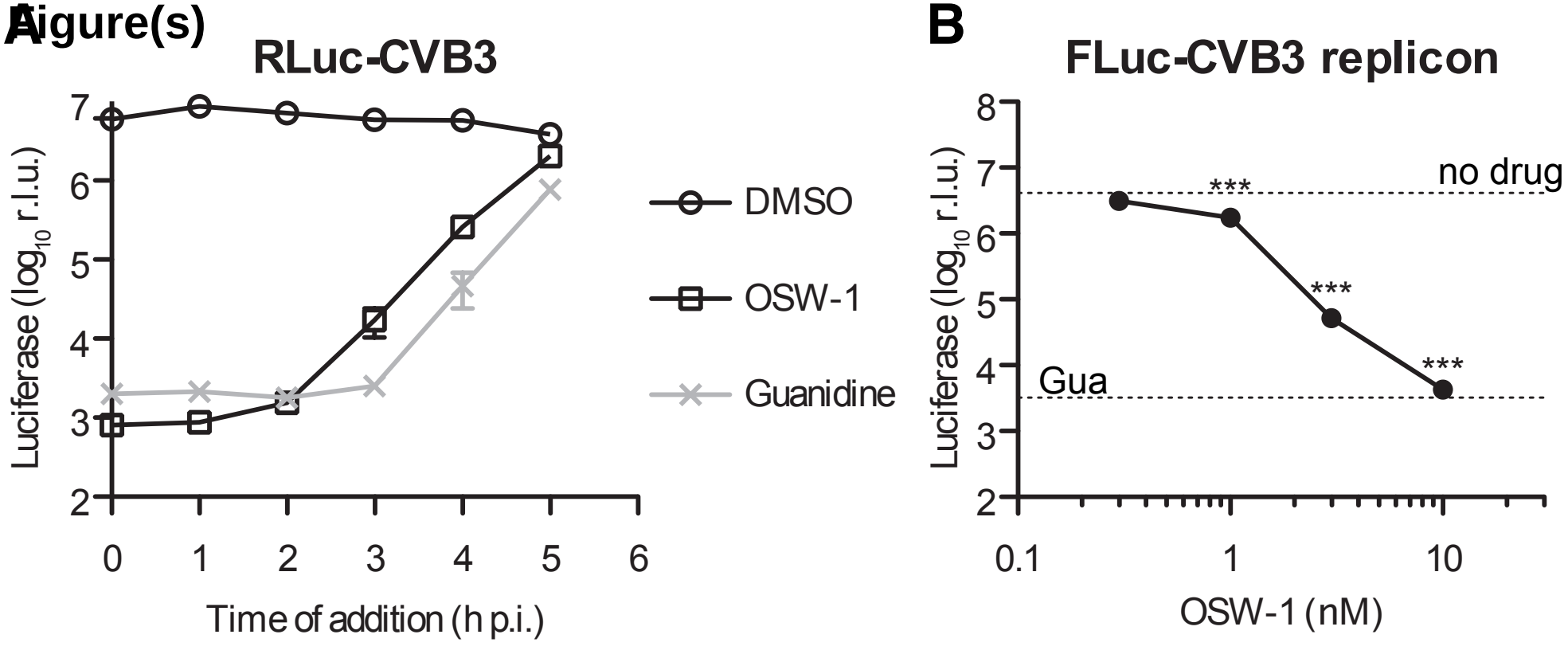
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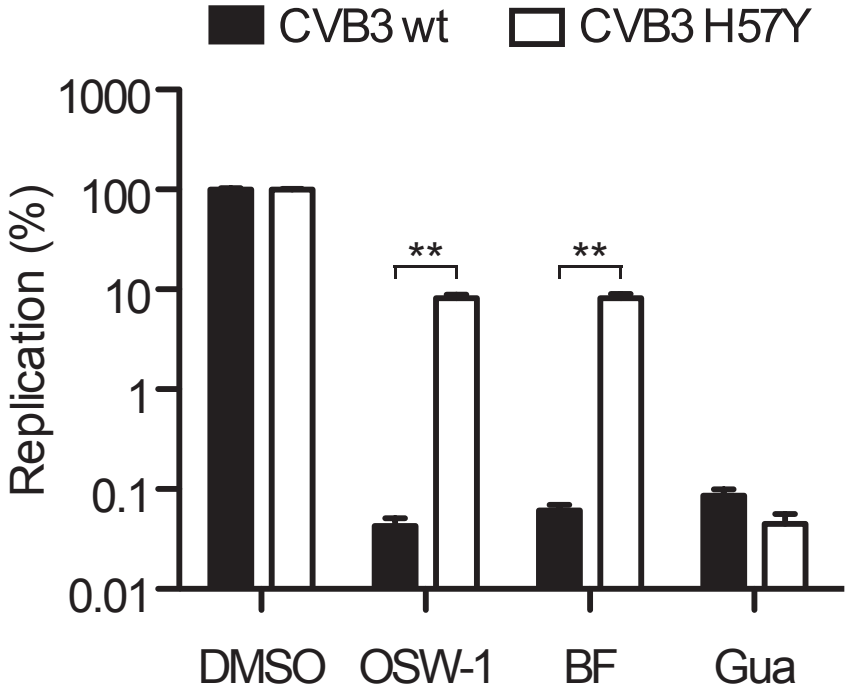
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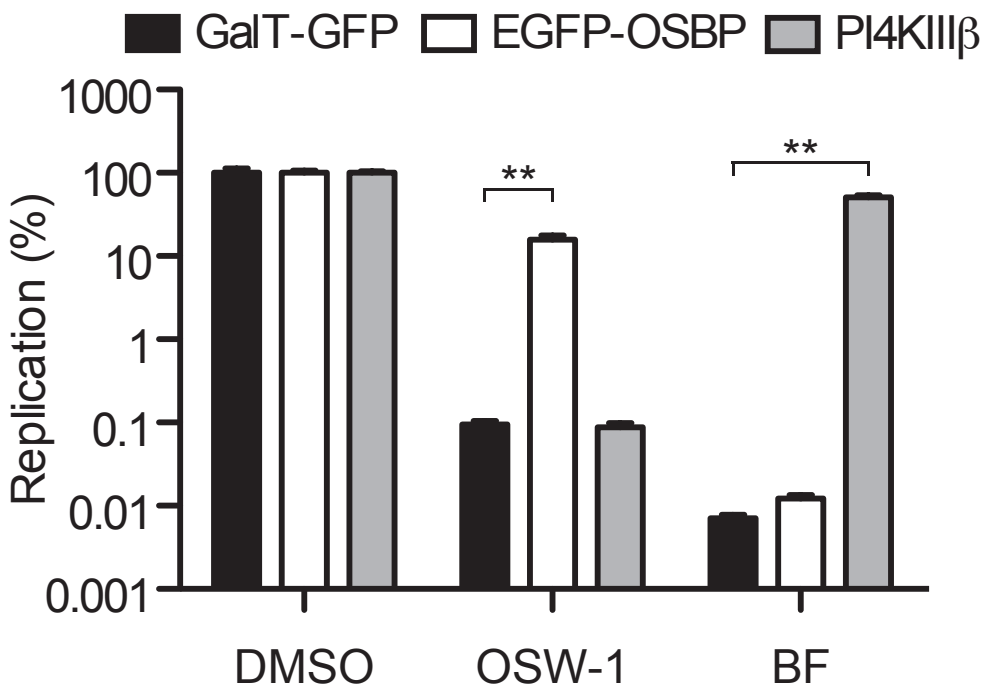
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