Broad-spectrum antiviral agents: secreted phospholipase A_2 targets viral envelope lipid bilayers derived from the endoplasmic reticulum membrane

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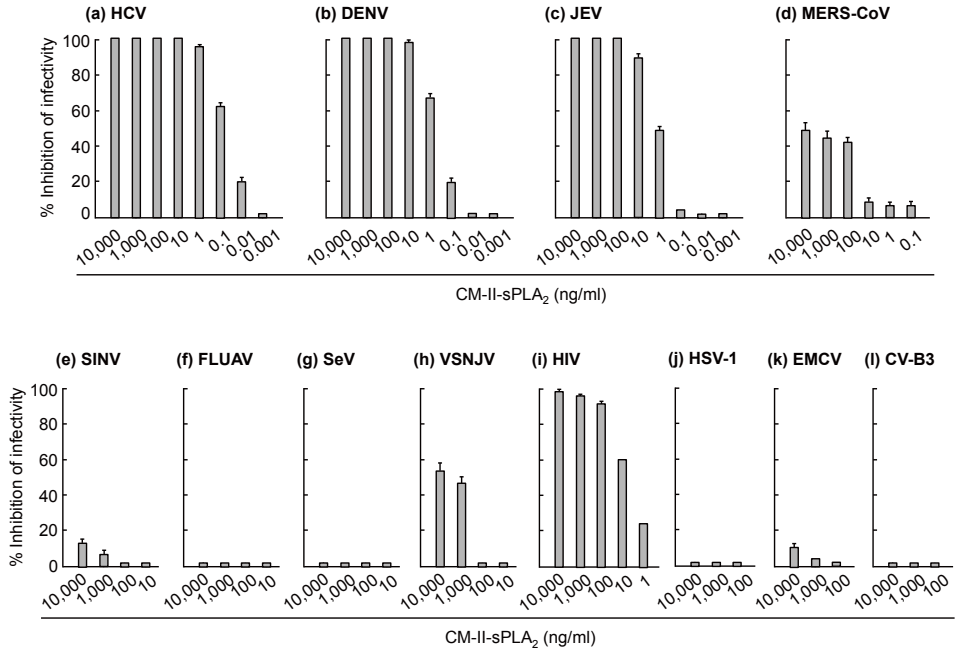
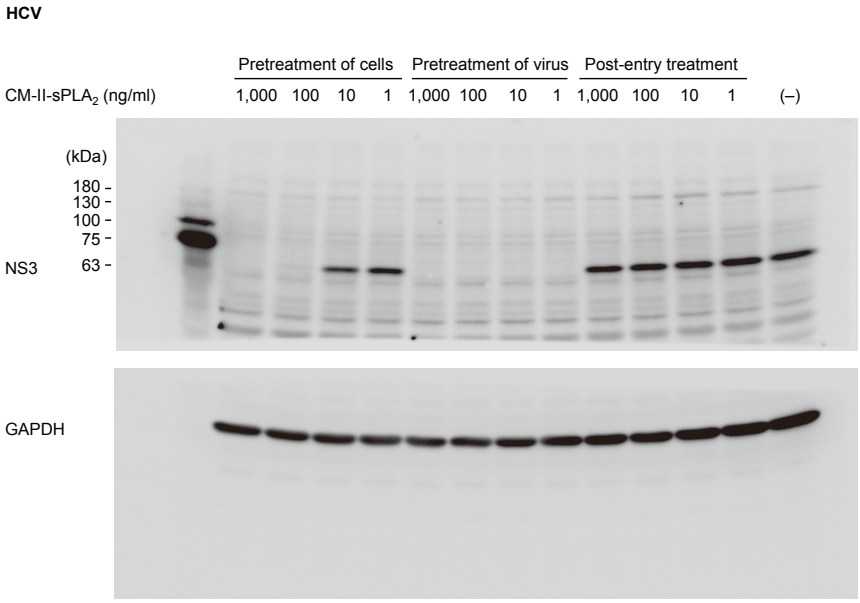
Supplementary Table S1. Time-of-addition experiments using CM-II-sPLA$_2$ against HCV and DENV.

Virus	CM-II-sPLA$_2$ (ng/ml)	% Reduction of the number of virus-infected cells		
		Pretreatment of cells	Pretreatment of virus	Post-entry treatment
HCV	1,000	93 ± 1	>99	<1
	100	54 ± 3	>99	<1
	10	16 ± 1	>99	<1
	1	4 ± 1	>99	<1
DENV	1,000	96 ± 1	>99	<1
	100	83 ± 5	>99	<1
	10	37 ± 3	>99	<1
	1	2 ± 2	>99	<1

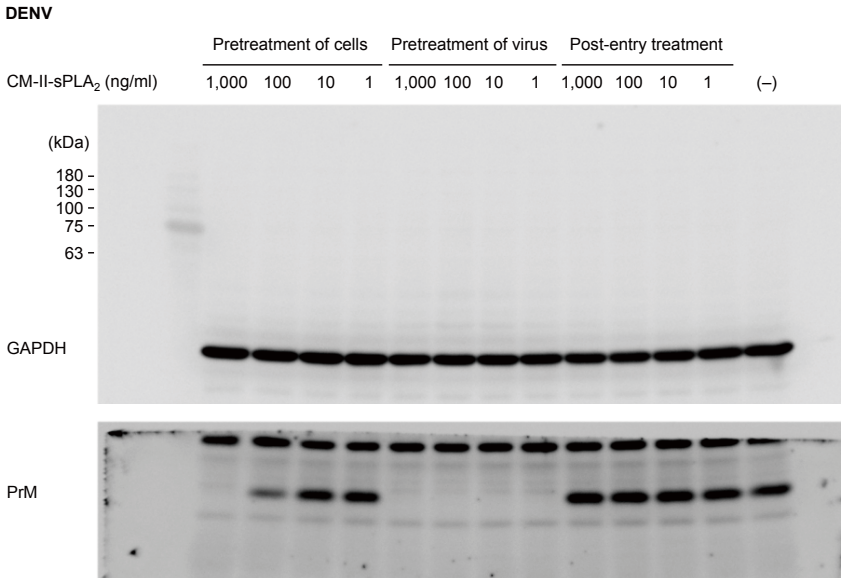
Data are presented as the average ± SEM (n = 2).



Supplementary Figure S1. Dose-dependent inhibition of a panel of viruses by CM-II-sPLA$_2$. A fixed amount of test virus was mixed with serial dilutions of sPLA$_2$s and incubated at 37°C for 1 h. The mixtures were inoculated onto the cells and incubated for another 1 h to allow virus adsorption. Then, the cells were washed with medium to remove the residual virus and cultured for 2 to 4 days (plaque assay and TCID$_{50}$ test), 24 h (FA test) or 2 days (TZM-bl assay) in fresh medium without sPLA$_2$s. Viral solutions not treated with sPLA$_2$s served as a control. The percent inhibition of viral infectivity by the sPLA$_2$s compared to the untreated control was calculated. Data are presented as the average ± SEM (n = 3 to 5).



Supplementary Figure S2. Time-of-addition experiments using CM-II-sPLA$_2$ against HCV. HCV NS3 expression levels were examined by immunoblotting analysis. (i) Pretreatment of the cells: Huh7it-1 cells were treated with various concentrations of CM-II-sPLA$_2$ (1,000, 100, 10 and 1 ng/ml) for 1 h. Then, the cells were inoculated with HCV in the absence of CM-II-sPLA$_2$ for another 1 h and cultured for 24 h in the absence of CM-II-sPLA$_2$. (ii) Pretreatment of the virus: HCV was incubated with CM-II-sPLA$_2$ for 1 h, and the mixtures were inoculated to Huh7it-1 cells. After 1 h, the cells were cultured for 24 h in the absence of CM-II-sPLA$_2$. (iii) Post-entry treatment: Huh7it-1 cells were inoculated with HCV in the absence of CM-II-sPLA$_2$. After 1 h, the cells were cultured for 24 h in the presence of CM-II-sPLA$_2$. (–), Untreated control. The lysates of the infected cells were subjected to 12% SDS-polyacrylamide gel electrophoresis and the full-length gel was electrophoretically blotted onto a polyvinylidene difluoride membrane. The membrane was cut into two pieces, which were probed with either anti-HCV NS3 (upper panel) or anti-GAPDH antibodies (lower panel). GAPDH bands serve as a loading control.



Supplementary Figure S3. Time-of-addition experiments using CM-II-sPLA$_2$ against DENV. DENV PrM expression levels were examined by immunoblotting. (i) Pretreatment of the cells: Huh7it-1 cells were treated with various concentrations of CM-II-sPLA$_2$ (1,000, 100, 10 and 1 ng/ml) for 1 h. Then, the cells were inoculated with DENV in the absence of CM-II-sPLA$_2$ for another 1 h and cultured for 24 h in the absence of CM-II-sPLA$_2$. (ii) Pretreatment of the virus: DENV was incubated with CM-II-sPLA$_2$ for 1 h, and the mixtures were inoculated onto Huh7it-1 cells. After 1 h, the cells were cultured for 24 h in the absence of CM-II-sPLA$_2$. (iii) Post-entry treatment: Huh7it-1 cells were inoculated with DENV in the absence of CM-II-sPLA$_2$. After 1 h, the cells were cultured for 24 h in the presence of CM-II-sPLA$_2$. (–), Untreated control. The lysates of the infected cells were subjected to 12% SDS-polyacrylamide gel electrophoresis and the full-length gel was electrophoretically blotted onto a polyvinylidene difluoride membrane. The membrane was cut into two pieces, which were probed with either anti-GAPDH (upper panel) or anti-DENV PrM antibodies (lower panel). GAPDH bands serve as a loading control.