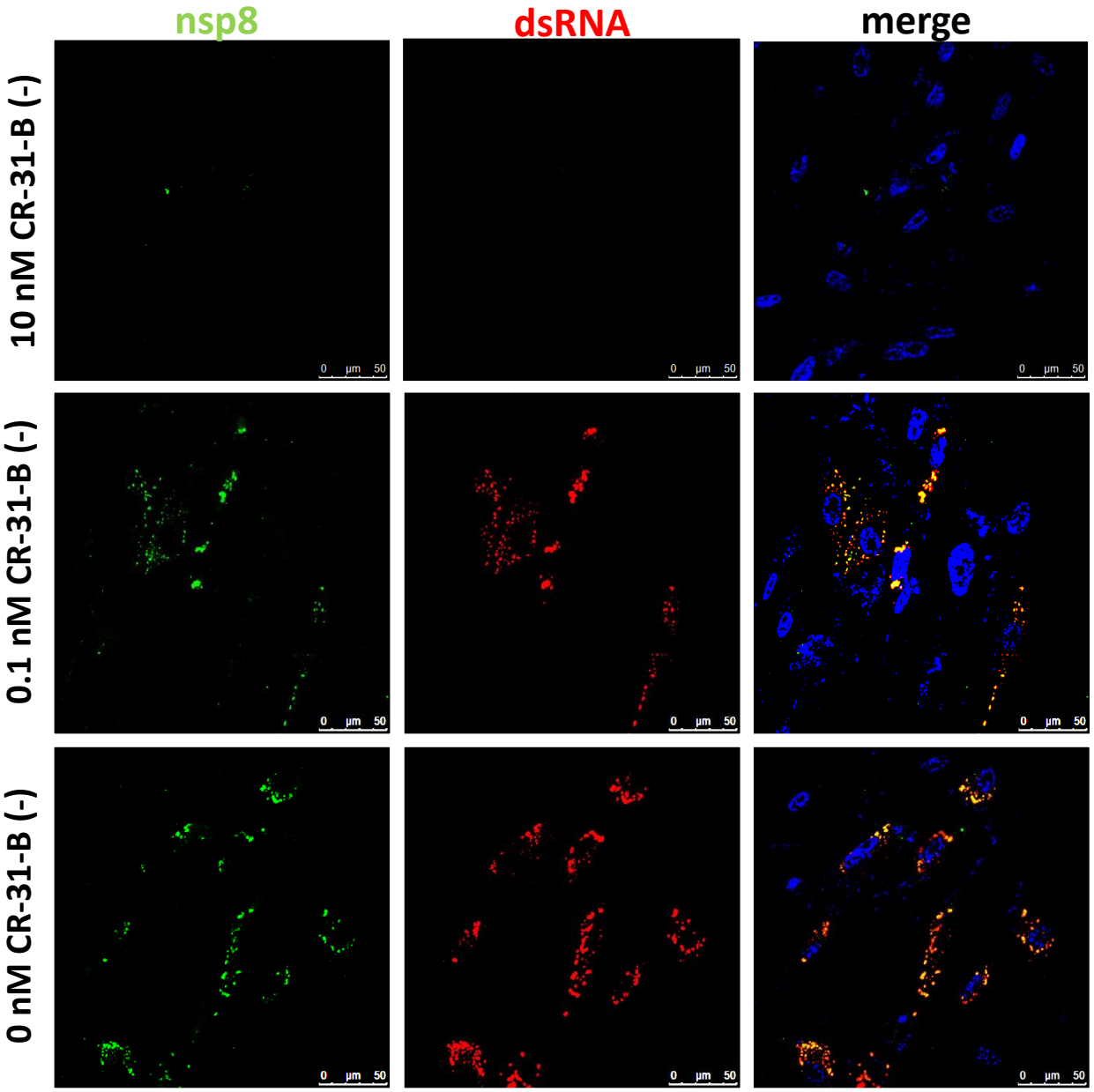
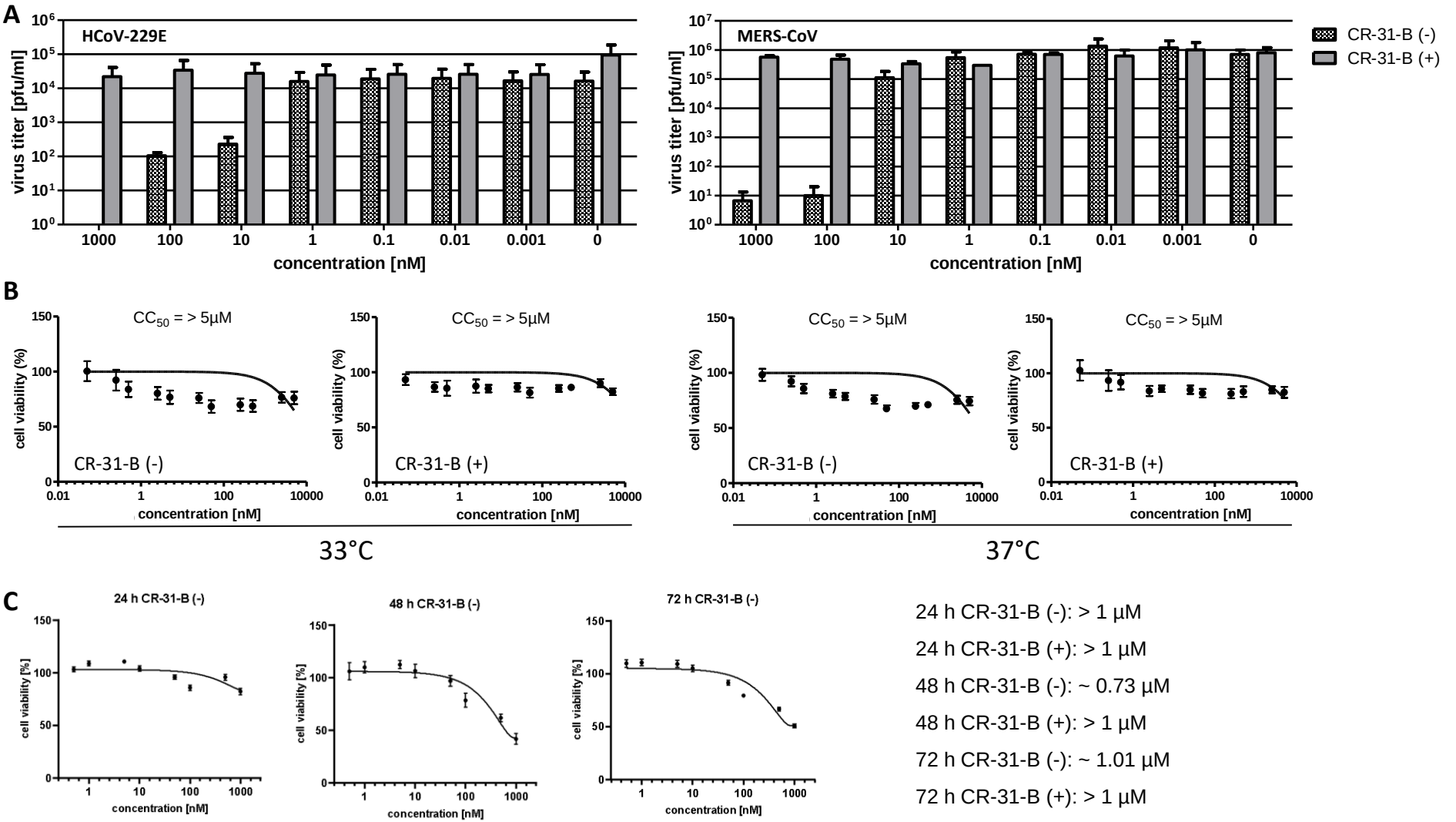


Immunofluorescence analysis to visualize the effects of CR-31-B (-) on viral dsRNA and nsp8 accumulation in HCoV-229E-infected MRC-5 cells. Cells were infected with an MOI of 1 and incubated with the indicated CR-31-B (-) concentrations (0 nM CR-31-B (-) means DMSO-treated cells). Cells were fixed at 24 h p.i. and analyzed by confocal laser-scanning microscopy using antibodies specific for dsRNA (red) and nonstructural protein 8 (nsp8, green).

Figure S1

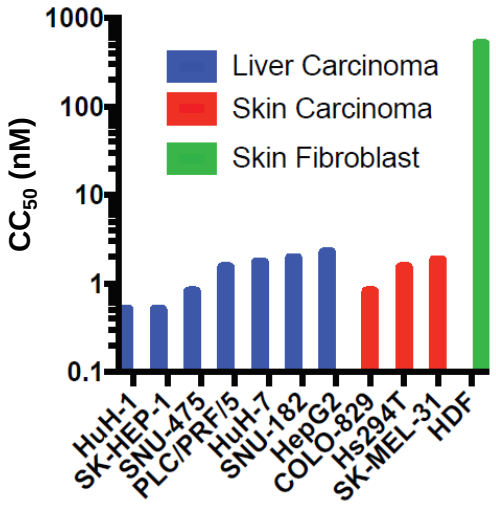


(A) CR-31-B (-) inhibits the production of infectious virus progeny of HCoV-229E and MERS-CoV at low nanomolar concentrations. **(B)** Treatment of MRC-5 cells for 24 h with CR-31-B (-) and CR-31-B (+) caused no major cytotoxicity at concentrations of up to 5 µM measured via MTT assay. **(C)** CC_{50} values determined via ATPlite assay for MRC-5 cells incubated with CR-31-B (-) or CR-31-B (+) for 24, 48 or 72 h as indicated.

Figure S2

CC_{50} values [nM] determined for the indicated cell types after treatment with CR-31-B for 48 h

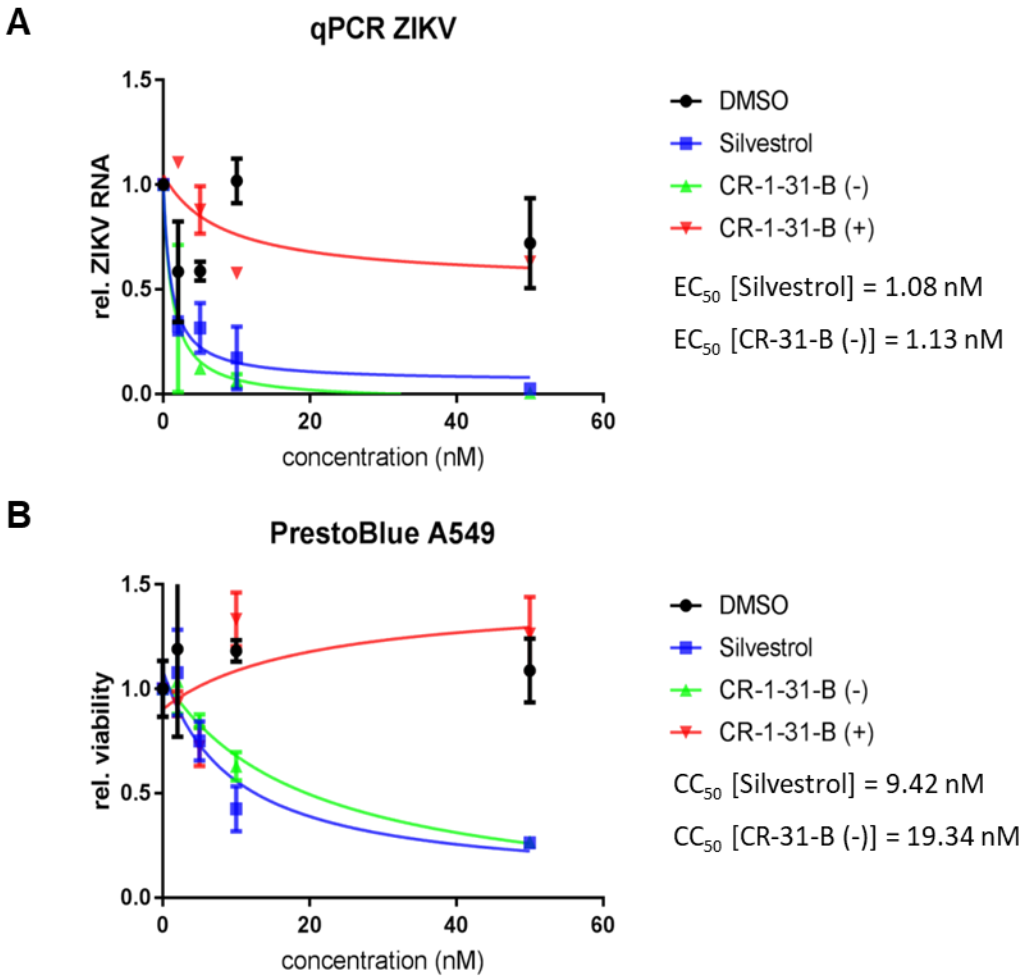
	Liver Carcinoma	Skin Carcinoma	Skin Fibroblast
HuH-1	0.5		
SK-HEP-1	0.5		
SNU-475	0.8		
PLC/PRF/5	1.5		
HuH-7	1.7		
SNU-182	1.9		
HepG2	2.2		
COLO-829		0.8	
Hs294T		1.5	
SK-MEL-31		1.8	
HDF			500



CC_{50} values were determined for a range of human skin carcinoma and liver carcinoma cell lines treated with racemic (+/-) CR-31-B. For comparison, the CC_{50} value of (+/-) CR-31-B was determined using primary human dermal fibroblasts (HDF). Data is representative of four experimental replicates. Average CC_{50} across the two biological replicates is plotted.

Method: Human dermal fibroblast and cancer cell lines were cultured as recommended by the American Type Culture Collection. Human dermal fibroblast cells were cultured in Fibroblast Basal Medium (ATCC® PCS201030™) supplemented with Fibroblast Growth Kit-Low Serum (ATCC PCS-201-041). Cancer cell lines were cultured in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/ml; 100 µg/ml). To determine CC_{50} values, cell lines were treated with racemic (+/-) CR-31-B for 48 hours and cell viability was measured by ATP quantification using the CellTiter-Glo Luminescent Cell Viability Assay (Promega G7571).

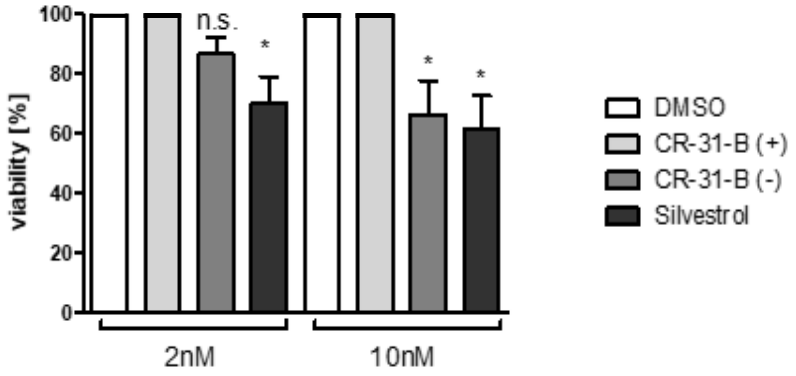
Figure S3



Potent antiviral activity of CR-31-B (-) and Silvestrol against the Zikavirus (Uganda strain) in A549 cells. EC_{50} values of both compounds are ~ 1 nM; CC_{50} values are 9.4 nM for Silvestrol and 19.3 for CR-31-B (-).

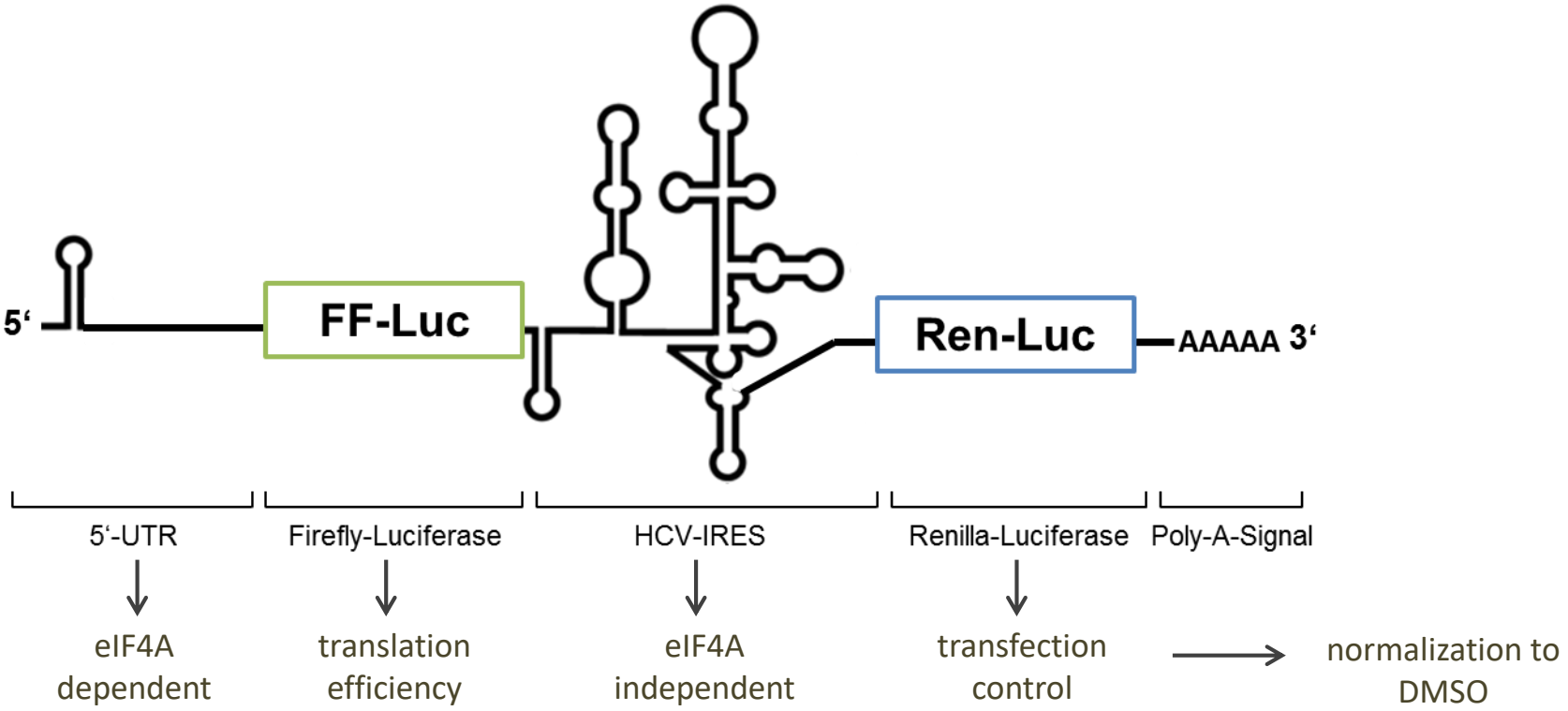
Methods: A549 cells were infected with the ZIKV strain 976 Uganda (U) (kindly provided by the European Virus Archive) using an MOI of 0.1 for 16 h. Simultaneously, the cells were treated with compounds in the respective concentrations. Afterwards, the inoculum was removed and cells were washed with PBS and treated with compounds for additional 8 h. Cells were lysed in peqGold Trifast 24 hpi to isolate intracellular RNA. After reverse transcription the ZIKV genomes were quantified by qRT-PCR to determine the EC50 as described above. Cell viability of A549 cells was determined using the PrestoBlue Cell viability reagent (Thermo Fisher Scientific) after treatment with the substances in the respective concentrations for 72 h.

Figure S4



Analysis of cytotoxicity of CR-31-B (+), CR-31-B (-) and Silvestrol in persistently HEV-infected A549 cells after treatment with the indicated compound for 72 h. At a concentration of 2 nM, both CR-31-B enantiomers had no major cytotoxic effects, whereas the natural rocaglate Silvestrol reduced the cell viability by approximately 30 %. At an increased concentration of 10 nM, both CR-31-B (-) and Silvestrol caused a reduction of cell viablity by approximately 40 %.

Figure S5



Scheme of the dual luciferase assay used to analyze the sensitivity of viral 5´-UTRs towards eIF4A inhibition

Figure S6