Figure 1

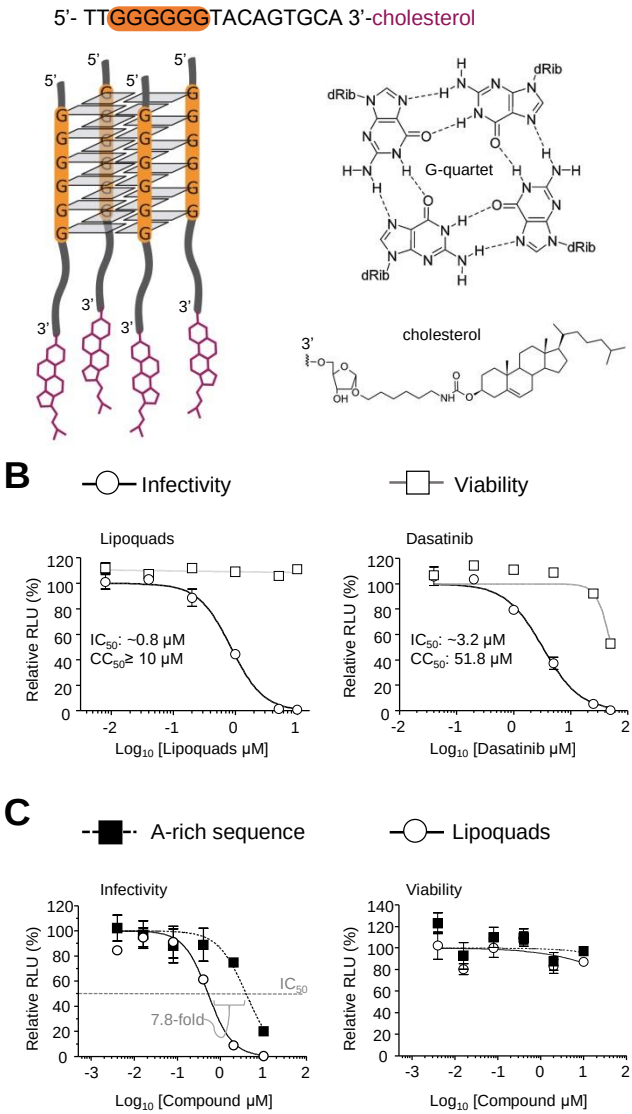

Figure 1. Quadruplex DNA structures (lipoquads) are potent HCV inhibitors. (A) Sequence and schematic structure of the parallel tetramolecular lipoquads of the present study, formed by the association of four 17-mer oligonucleotides containing the GGGGGG (orange) repeat. The structure is held by six stacked G-quartets. The 3' extremities of the individual strands are conjugated with a cholesteryl function (magenta), forming a lipophilic tail. (B) Anti-HCV activity of lipoquads and viability of Huh7/Scr cells infected with Luc-Jc1 HCVcc viruses (at an MOI 0.01-0.03 $TCID_{50}$/cell) and treated with increasing concentrations of lipoquads or dasatinib (positive control). (C) Anti-HCV activity and viability on Huh7/Scr cells infected with Luc-Jc1 HCVcc viruses and treated with increasing concentrations of lipoquads or A-rich control sequence. Infectivity and viability were determined 72h post infection by luciferase assays. Results are the means (± SEM) from two replicate infections measured in duplicates and expressed as relative RLU compared to the infection of control (mock) cells. The data presented are from a single experiment and are representative of three independent experiments.

Figure 2

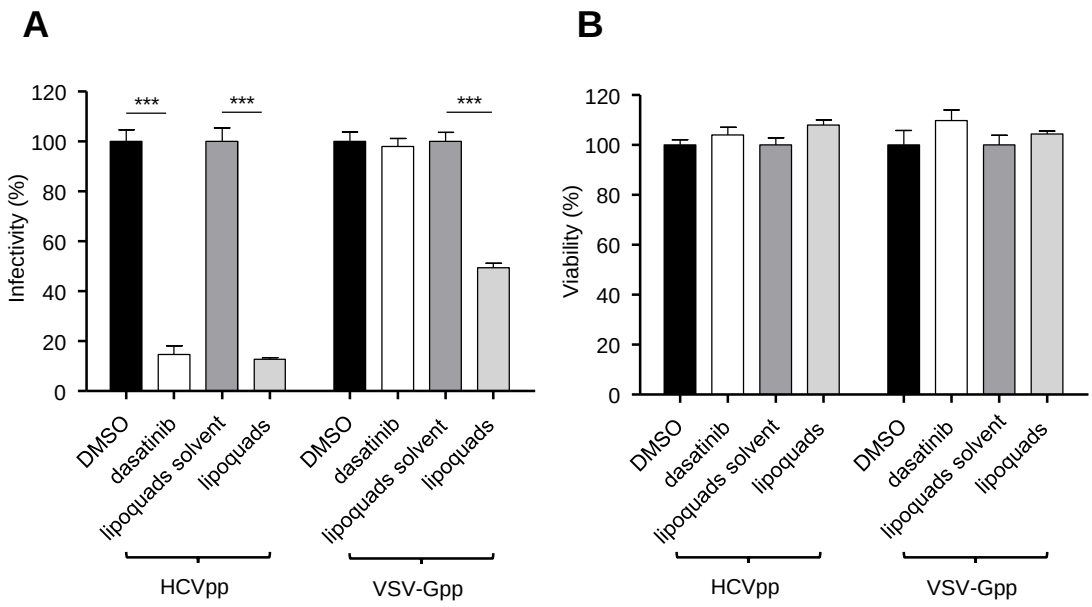

Figure 2. Lipoquads preferentially inhibit HCV pseudoparticles (HCVpp). Huh7/Scr cells were pretreated for 1h with 5 µM of lipoquads or dasatinib. Then, cells were inoculated with HCVpp or VSV-Gpp in the presence of compounds. Six hours later medium was replaced with fresh media-compounds mix and cells. Infectivity and viability were determined 72h post infection by *Renilla* luciferase for infectivity (A) or *Firefly* luciferase assays for cell viability (B). Results are the means (± SEM) from two replicate infections measured in duplicates and expressed as relative RLU compared to the infection of control (lipoquads solvent for lipoquads or DMSO for dasatinib) cells for both infectivity and viability. The data presented are from a single experiment and are representative of three independent experiments.

Figure 3

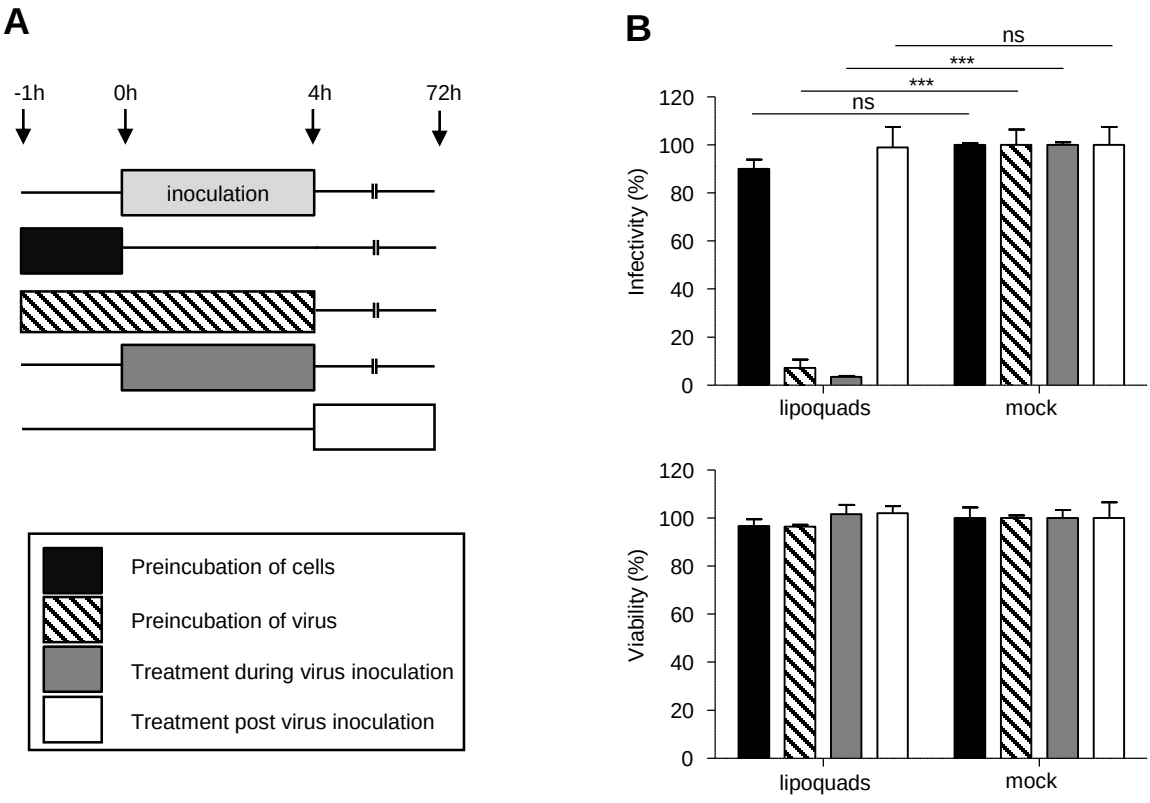

Figure 3. Lipoquads interact with HCV particles and selectively inhibit early HCV entry events. Huh7/Scr cells were inoculated with Luc-Jc1 HCVcc viruses (at an MOI 0.01-0.03 $TCID_{50}$/cell) prepared in the absence of drugs. Lipoquads (final concentration 1 µM) were added to the cells only before inoculation for 1h (black), pre-incubated with the viruses (stripes), only during inoculation (grey) or selectively after infection (white), as schematically depicted in (A). Infectivity and viability (B) were determined 72h later by *Firefly* luciferase assays. Results are the means (± SEM) from two replicate infections measured in duplicates and expressed as relative RLU compared to the infection of control (mock) cells. The data presented are from a single experiment and are representative of three independent experiments.

Figure 4

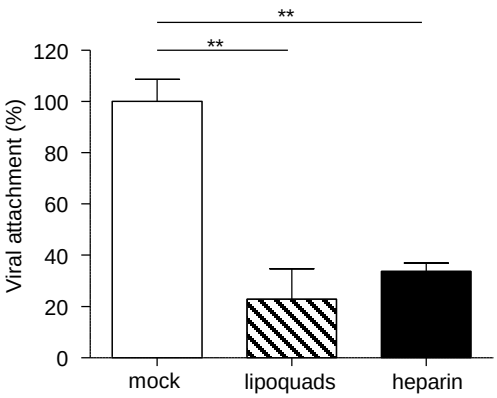

Figure 4. Lipoquads block virus attachment to the cell. Huh7/Scr cells were placed on ice for 30 min to cool down. Cells were then incubated for 2h with pre-chilled Jc1 HCVcc virus (MOI ~10 $TCID_{50}$/cell) at 4ºC. Lipoquads (5 µM) or heparin (100 µg/ml) or buffer (mock) were added to the cells simultaneously to virus inoculation. Cell monolayers were washed 3 times with cold PBS and then lysed and subjected to RNA extraction. HCV RNA was quantified by qRT-PCR in 25 ng of total RNA. Results are the means (± SEM) from two replicate infections measured in triplicates and expressed as relative RNA compared to the viral attachment of control (mock) cells. The data presented are from a single experiment and are representative of three independent experiments.

Figure 5

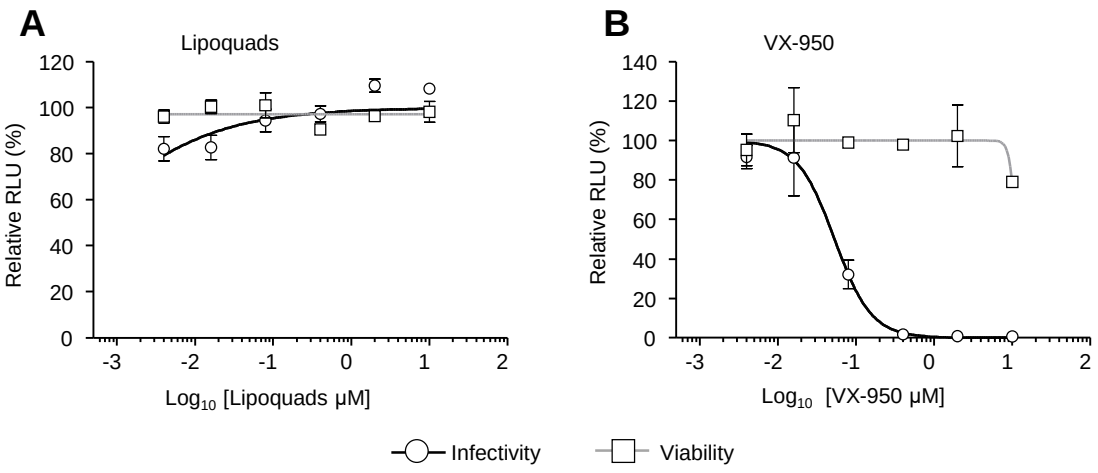

Figure 5. Lipoquads do not inhibit HCV subgenomic replicons. Huh7/Scr cells were pre-treated with lipoquads or telaprevir (VX-950) at increasing concentrations (0.004, 0.016, 0.08, 0.4, 2 and 10 µM) for 1h, transfected with SGR RNA (compounds present) and seeded in 96-well plates. Four-hours post transfection lipofectamine-RNA-containing media was replaced by a fresh media-compounds mix and *Firefly* luciferase activity was assayed 24h later. In both A and B results are the means (± SEM) from two replicate transfections measured in duplicates and expressed as relative RLU compared to the transfection of control (mock) cells for both infectivity and viability. The data presented are from a single experiment and are representative of three independent experiments.

Figure 6

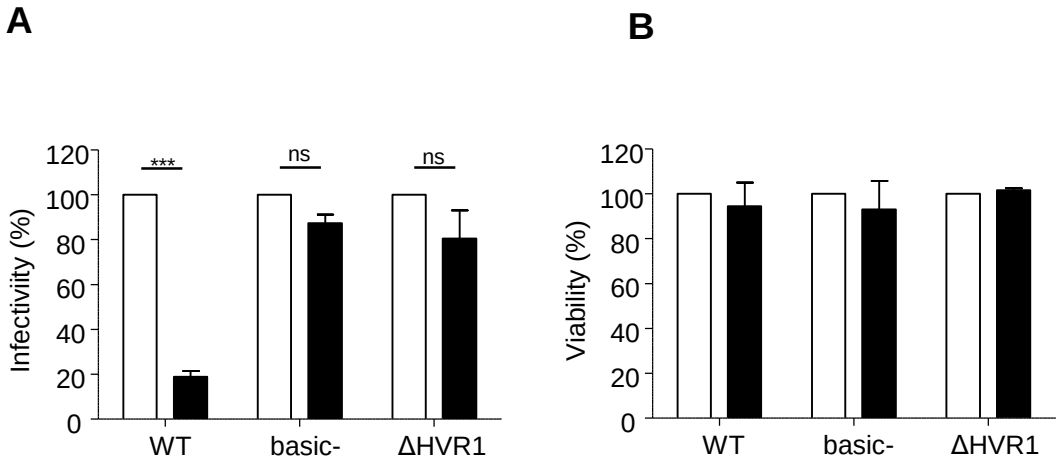

Figure 6. Basic aminoacis in E2 HVR1 and HVR1 itself play a role in lipoquad-mediated HCV inhibition. Huh7/Scr cells were inoculated with Luc-Jc1 WT HCVcc viruses or with the indicated mutants (at an MOI 0.01-0.03 $TCID_{50}$/cell). Lipoquads (final concentration 1 μM) were added to the cells simultaneously to inoculation. HCVcc-compounds mix were replaced 4h post infection with fresh media-compounds mix and 72h after infection cells were assayed for *Firefly* luciferase activity. Results are the means (± SEM) from two replicate infections measured in duplicates (black bars) and expressed as relative RLU compared to the infection of control (mock) cells (white bars) for both infectivity (A) and viability (B). The data presented are from a single experiment and are representative of three independent experiments.

Figure 7

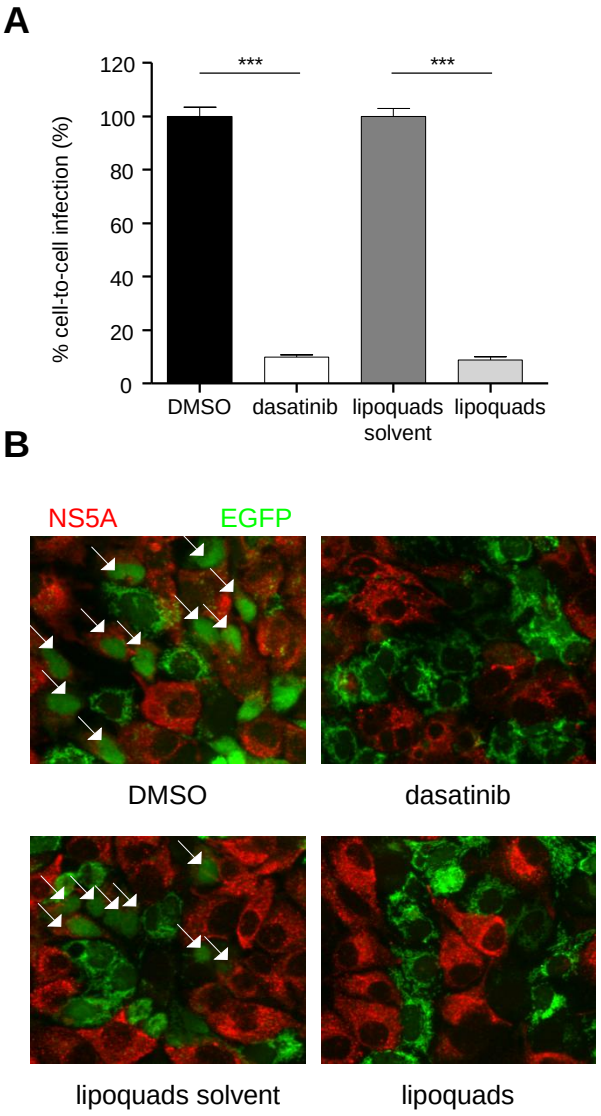

Figure 7. Lipoquads inhibit HCV cell-to-cell transmission. Huh7/Scr cells were infected with Jc1 HCVcc virus at an MOI ~10 $TCID_{50}$/cell and 20h later seeded on 24 well plates. Simultaneously, Huh7.5/EGFP-NLS-IPS cells were seeded on the same 24 well plates (co-culture, at a ratio 1:1 with Huh7/Scr cells) and lipoquads (10 μM) or dasatinib (10 μM) were added to the wells. The addition of the compounds solvent (lipoquads solvent or DMSO) served as negative control. Four hours later, medium was removed and cells were overlaid with 1% agarose with fresh compounds. Twenty four hours later, the HCV infection was analysed by immunofluoresce of NS5A staining. (A) Results are the means of quantification of Huh7.5/EGFP-NLS-IPS cells in 3 independent wells by taking 3 independent pictures of different fields of each well that contained at least 200 cells in total. Cell-to-cell inhibition is expressed as the percentage of acceptor cells with the EGFP-NLS + NS5A signal with respect to the total number of EGFP + NS5A cells in the DMSO (for dasatinib) or lipoquads buffer (for lipoquads) treated wells. (B) Representative images of co-culture cells stained with anti-NS5A antibodies. Huh7.5/EGFP-NLS-IPS cells with EGFP signal redestributed to the nucleus and cytoplasmatic NS5A signal are delineated with arrows. Magnification 63x.

Figure 8

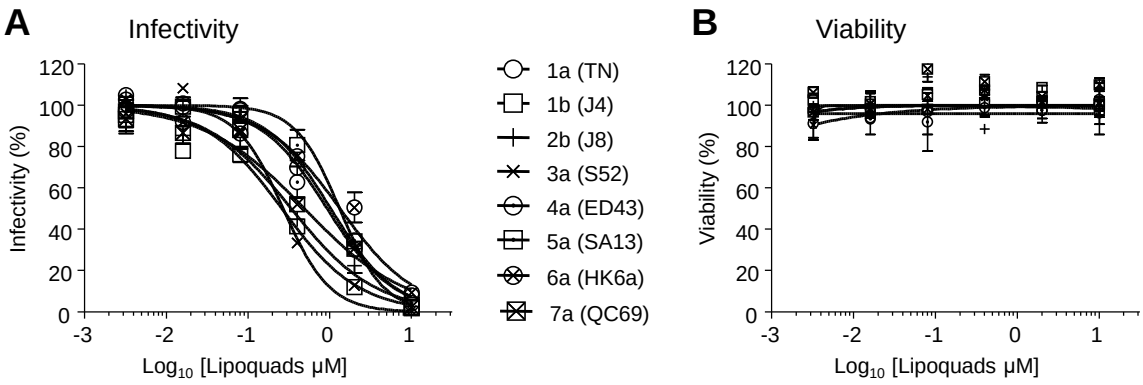

Figure 8. Lipoquads inhibits HCV of different genotypes. Huh7/Scr cells were inoculated with viruses of the indicated genotypes and isolates (at an MOI ~0.01-0.03 $TCID_{50}$/cell). Lipoquads in the indicated concentrations were added to the cells simultaneously to inoculation. HCVcc-compounds mix were replaced 4h post infection with fresh media-compound mix. Seventy two hours post infection cells were assayed for *Renilla* luciferase activity for infectivity (A) or *Firefly* luciferase for cell viability (B). Results are the means (± SEM) from two replicate infections measured in duplicates and expressed as relative RLU compared to the infection of control (mock) cells. The data presented are from a single experiment and are representative of three independent experiments.

Figure 9

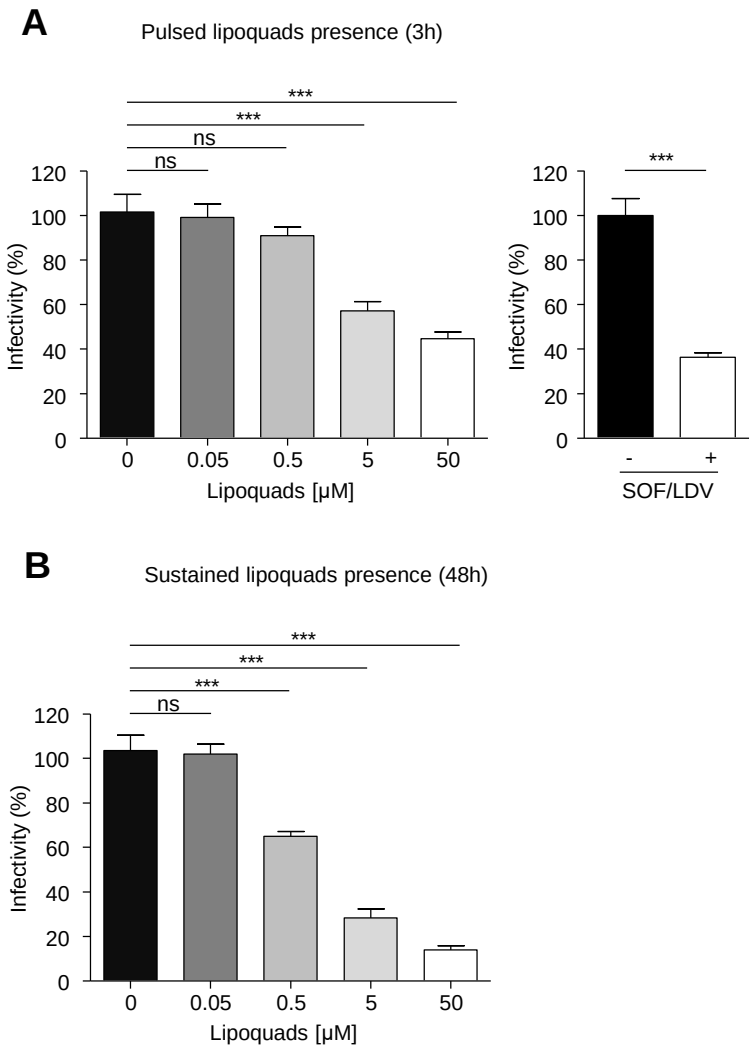

Figure 9. Inhibitory activity of lipoquads in colorectal tissue explants against HCVcc Jc1FLAG(p7-nsGluc2A). Mucosal explants were treated for 1h in the presence or absence of lipoquads (at the indicated concentrations) or SOF/LDV (both at 1 µM final concentration) prior to viral exposure for 2h (total compounds presence 3h). Explants were then washed four times with PBS and transferred to gel foam rafts. Tissue explants were kept in culture for 48h in the absence (pulse) (A) or in the sustained compounds presence (B). Culture supernatants were harvested for detection of *Gaussia* luciferase activity and the extent of infection was plotted as percentage relative to the relative light units (RLU) obtained for explants infected with virus in the absence of compound (100% infectivity). Data represent the means of three independent experiments performed in triplicates (±SEM).