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Supplemental Information

A Trans-amplifying RNA Vaccine Strategy for Induction of Potent Protective Immunity

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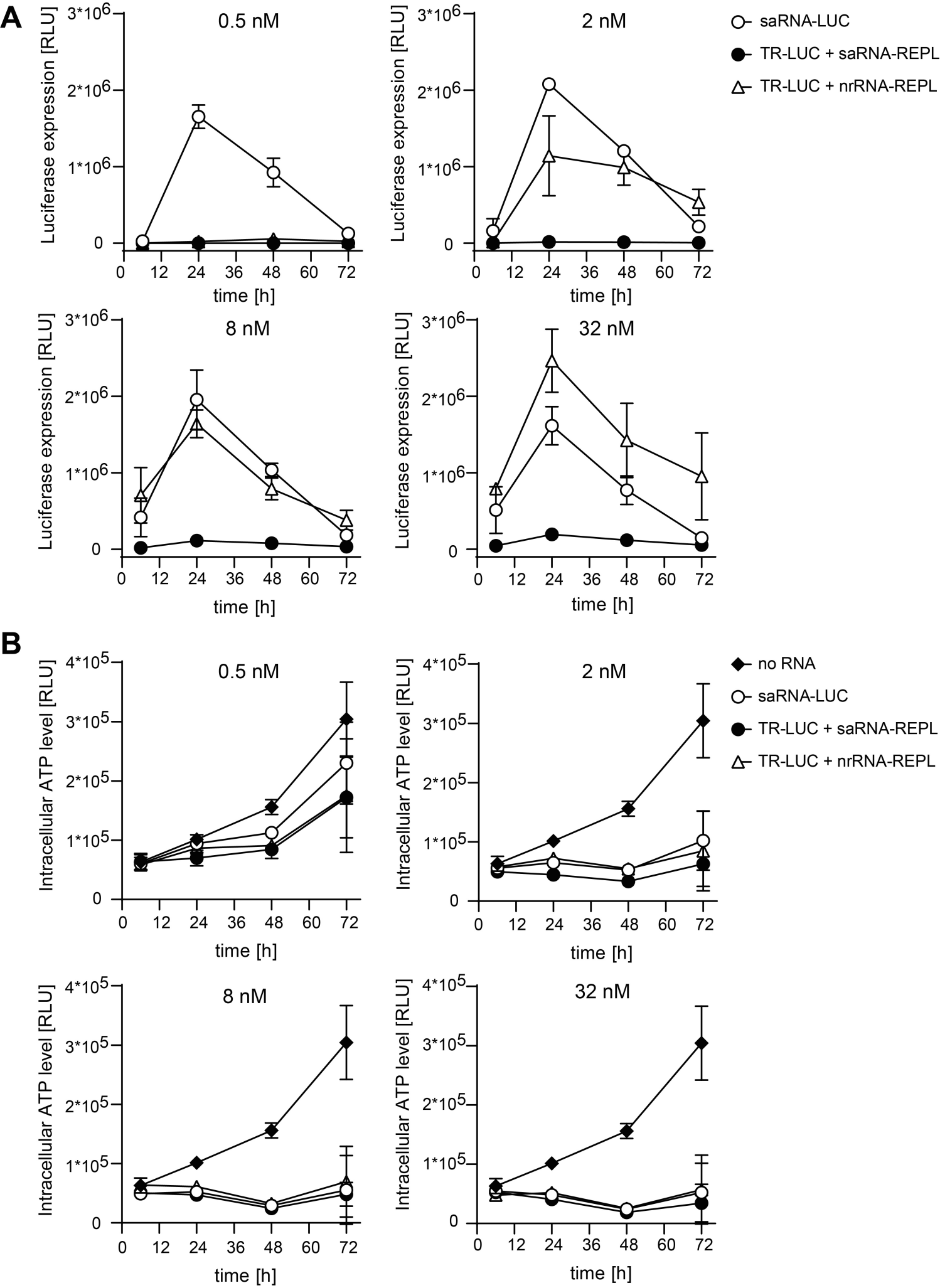
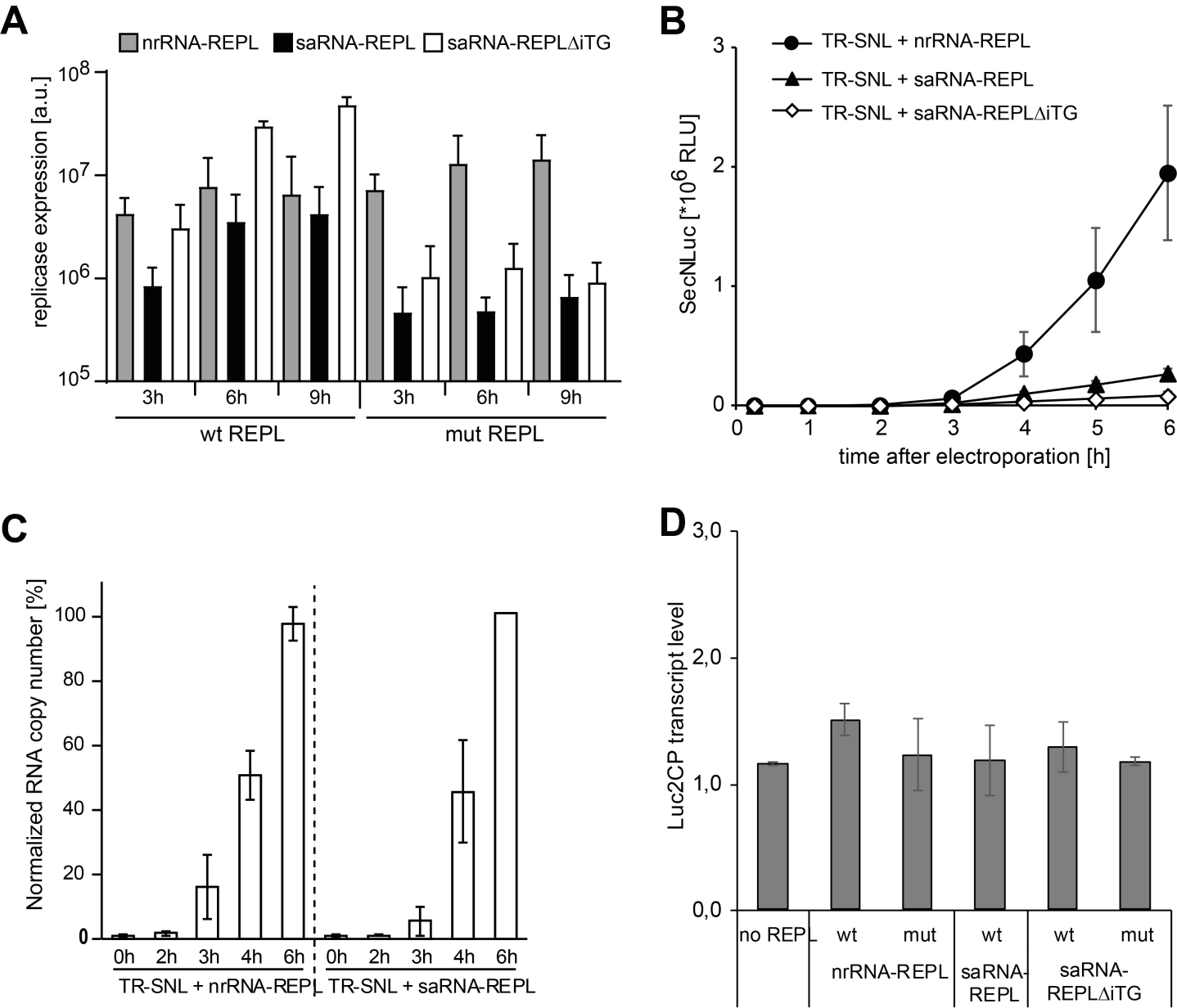
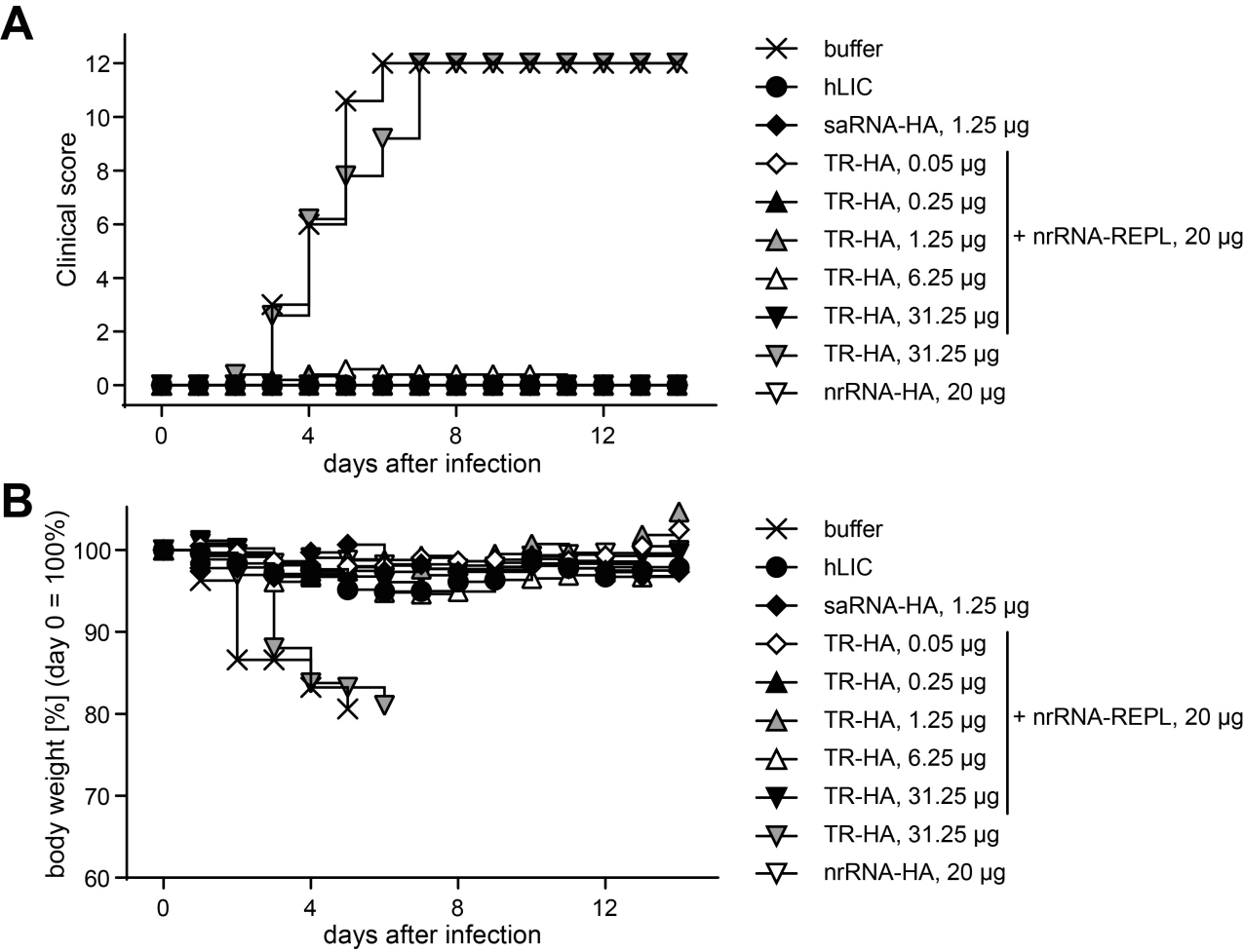

A
0.5 nM
2 nM
8 nM
32 nM
Luciferase expression [RLU]
time [h]
saRNA-LUC
TR-LUC + saRNA-REPL
TR-LUC + nrRNA-REPL
B
Intracellular ATP level [RLU]
no RNA
saRNA-LUC
TR-LUC + saRNA-REPL
TR-LUC + nrRNA-REPL

Supplemental figure 1. Expression from taRNA in conjunction with nrRNA-delivered replicase activity is as efficient as expression from self-amplifying RNA. Kinetics and raw data used for calculating areas under curve in Figure 1**.** Vectors encoding firefly luciferase (LUC) as GOI were electroporated into BHK21 cells ($3*10^6$ cells/ sample) in equimolar amounts (indicated in the upper left of each panel) and **(A)** Luciferase expression and **(B)** cell viability were measured. Mean and standard deviation of 3 independent experiments are shown.


A
nrRNA-REPL
saRNA-REPL
saRNA-REPLΔiTG
replicase expression [a.u.]
10^8
10^7
10^6
10^5
3h
6h
9h
3h
6h
9h
wt REPL
mut REPL
B
TR-SNL + nrRNA-REPL
TR-SNL + saRNA-REPL
TR-SNL + saRNA-REPLΔiTG
SecNLuc [*10^6 RLU]
3
2
1
0
0
1
2
3
4
5
6
time after electroporation [h]
C
Normalized RNA copy number [%]
100
80
60
40
20
0
0h
2h
3h
4h
6h
0h
2h
3h
4h
6h
TR-SNL + nrRNA-REPL
TR-SNL + saRNA-REPL
D
Luc2CP transcript level
3,0
2,0
1,0
0,0
no REPL
wt
mut
wt
mut
wt
mut
nrRNA-REPL
saRNA-REPL
saRNA-REPLΔiTG

Supplemental figure 2. taRNA expression and TR amplification early after transfection. **(A) Intracellular replicase protein levels.** $5*10^6$ BHK-21 cells per sample were co-electroporated with a TR encoding secreted Nano®-Luciferase (TR-SNL)(3.2 nM) , and 32 nM either nrRNA-REPL, saRNA-REPL without iTG or saRNA-REPL encoding luciferase as iTG. Cells were lysed after 3 h, 6 h, and 9 h to assess replicase expression by Western blot against the nsP3-myc tag. Densitometric analysis of specific bands is shown to compare expression. **(B) Early expression of TR vectors.** $1.5*10^6$ BHK21 cells were co-electroporated with 3.2 nM TR-SNL and 32 nM of either nrRNA-REPL, saRNA-REPL or saRNA-REPLΔiTG. Culture supernatants were collected to quantify SecNLuc accumulation. **(C) Analysis of TR amplification after transfection.** $1.5*10^6$ BHK-21 cells were electroporated with 3.2 nM TR-SNL and 32 nM of either nrRNA-REPL or saRNA-REPL. Cells were harvested and used for RNA isolation and cDNA synthesis at the indicated timepoints. qPCRs were performed using SNL-specific primers. Since Ct values differed experiments the data were normalized to the 6 h RNA-level of the sample "TR-SNL + saRNA-REPL". A to C: Mean and SD of three independent experiments. **(D) Quantification of a cell-made reporter transcript.** BHK21 cells were generated by lentiviral transduction to express destabilized luciferase (Luc2CP). $0.5*10^6$ transduced BHK-21 cells per sample were co-electroporated with a TR encoding SecNLuc as iTG (3.2 nM), and 32 nM of either nrRNA-REPL, saRNA-REPL without iTG or saRNA-REPL encoding GFP as iTG. Luc2CP transcript level was assessed 6h after RNA electroporation and normalized to the transcript level in cells electroporated without RNA (mean and SD of two independent experiments).


A
Clinical score
12
10
8
6
4
2
0
0
4
8
12
days after infection
buffer
hLIC
saRNA-HA, 1.25 µg
TR-HA, 0.05 µg
TR-HA, 0.25 µg
TR-HA, 1.25 µg
TR-HA, 6.25 µg
TR-HA, 31.25 µg
+ nrRNA-REPL, 20 µg
TR-HA, 31.25 µg
nrRNA-HA, 20 µg
B
body weight [%] (day 0 = 100%)
100
90
80
70
60
0
4
8
12
days after infection
buffer
hLIC
saRNA-HA, 1.25 µg
TR-HA, 0.05 µg
TR-HA, 0.25 µg
TR-HA, 1.25 µg
TR-HA, 6.25 µg
TR-HA, 31.25 µg
+ nrRNA-REPL, 20 µg
TR-HA, 31.25 µg
nrRNA-HA, 20 µg

Supplemental figure 3. Clinical score and body weight of vaccinated mice upon challenge infections. BALB/c mice were vaccinated by intradermal injection of TR-HA. To drive replication, 20 µg nrRNA-REPL was co-injected. Reference groups received either 20 µg nrRNA-HA or 1.25 µg saRNA-HA, negative controls received the highest amount of TR-HA without nrRNA-REPL or 20 µl buffer without RNA. Intramuscular injection of 2.4 HA-units human licenced vaccine (hLIC) served as positive control. Vaccination was performed as prime-boost regimen (day 0 and day 21). Challenge infections with 10-fold LD50 of viable Influenza A/California/4/2009 (H1N1) were done on day 56. **(A)** Clinical score of infected animals, **(B)** body weight of infected animals (mean of groups of 5 animals are shown).

Supplemental methods:

Quantitative real-time reverse transcriptase PCR (qRT-PCR). To analyse amplification of trans-replicons or the transcript levels of Luc2CP in transduced cells, total cellular RNA was extracted using the RNeasy Mini Kit (Qiagen) according the manufacturer's instructions and quantified by spectroscopy (NanoDrop 2000c, PeqLab) and reverse transcribed with Superscript II reverse transcriptase (Invitrogen) using random hexamers for first strand synthesis. qRT-PCR were performed in triplicates using the QuantiTect SYBR Green PCR Kit (Qiagen), the ABI 7300 Real time PCR System, and the companion SDS analysis software (Applied Biosystems), following the manufacturer's instruction. Analysis was performed using the $2^{-\Delta\Delta CT}$ method[1], normalized to the housekeeping gene HPRT. The following specific primers and annealing temperatures were used for amplification: HPRT, forward: 5' TGACACTGGCAAAACAATGCA-3', revers: 5'-GGTCCTTTTCACCAGCAAGCT-3 (60°C); SecNLuc, forward: 5'-CTGGACCAAGTCCTTGAAC-3', revers: 5'-CGCTCAGACCTTCATACG-3' (60°C); Luc2CP, forward: 5'-CCCATCTTCGGCAACCAGAT-3', revers: 5'-GTACATGAGCACGACCCGAA-3' (62°C).

References

1 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25, 402–408.