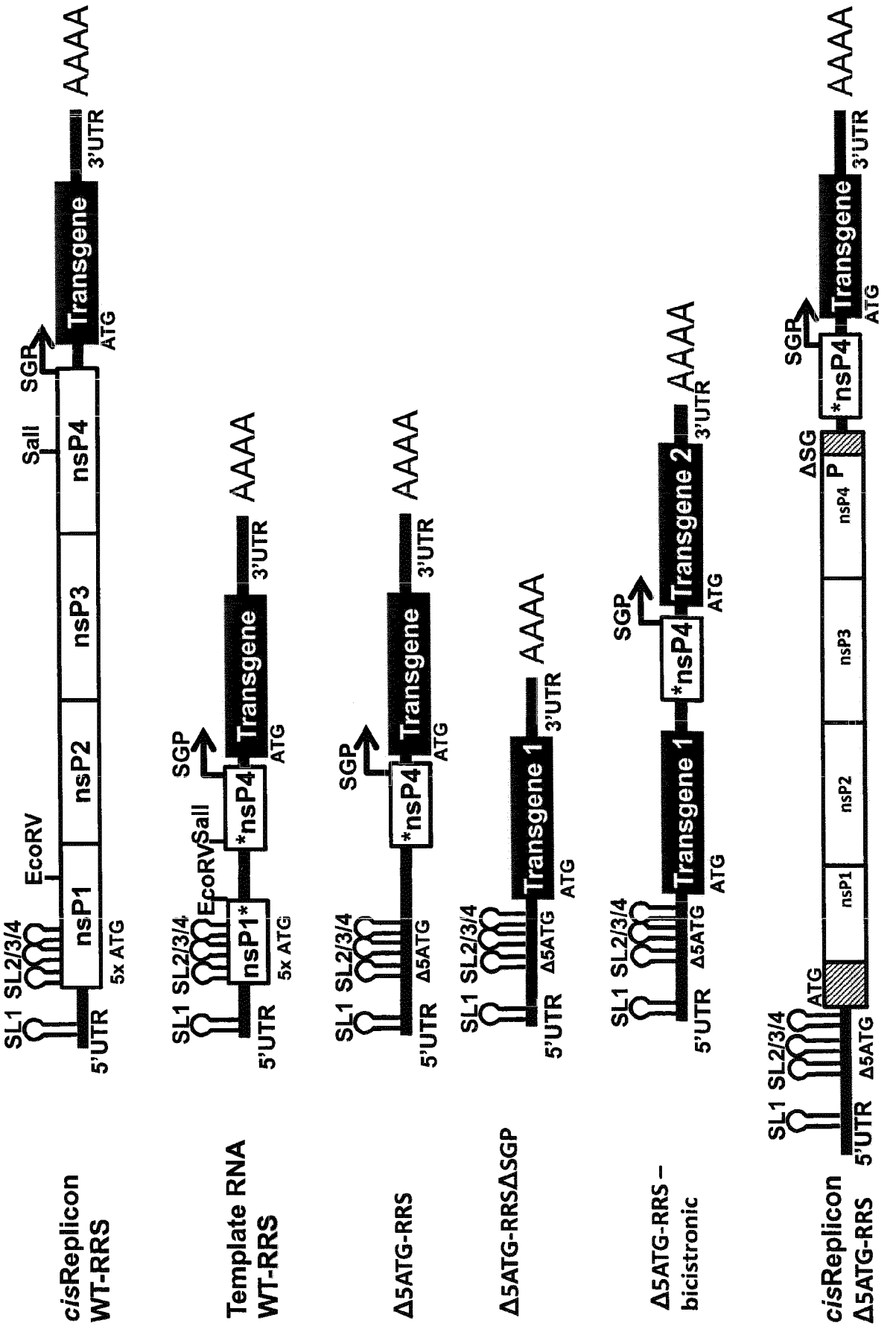

cisReplicon WT-RRS
5'UTR
SL1
SL2/3/4
nsP1
5x ATG
EcoRV
nsP2
nsP3
SalI
nsP4
SGR
Transgene
ATG
3'UTR
AAAA
Template RNA WT-RRS
*nsP1
*nsP4
Δ5ATG-RRS
Δ5ATG
Δ5ATG-RRSΔSGP
Transgene 1
Δ5ATG-RRS – bicistronic
SGP
Transgene 2
cisReplicon Δ5ATG-RRS
ΔSG
P

Fig. 1

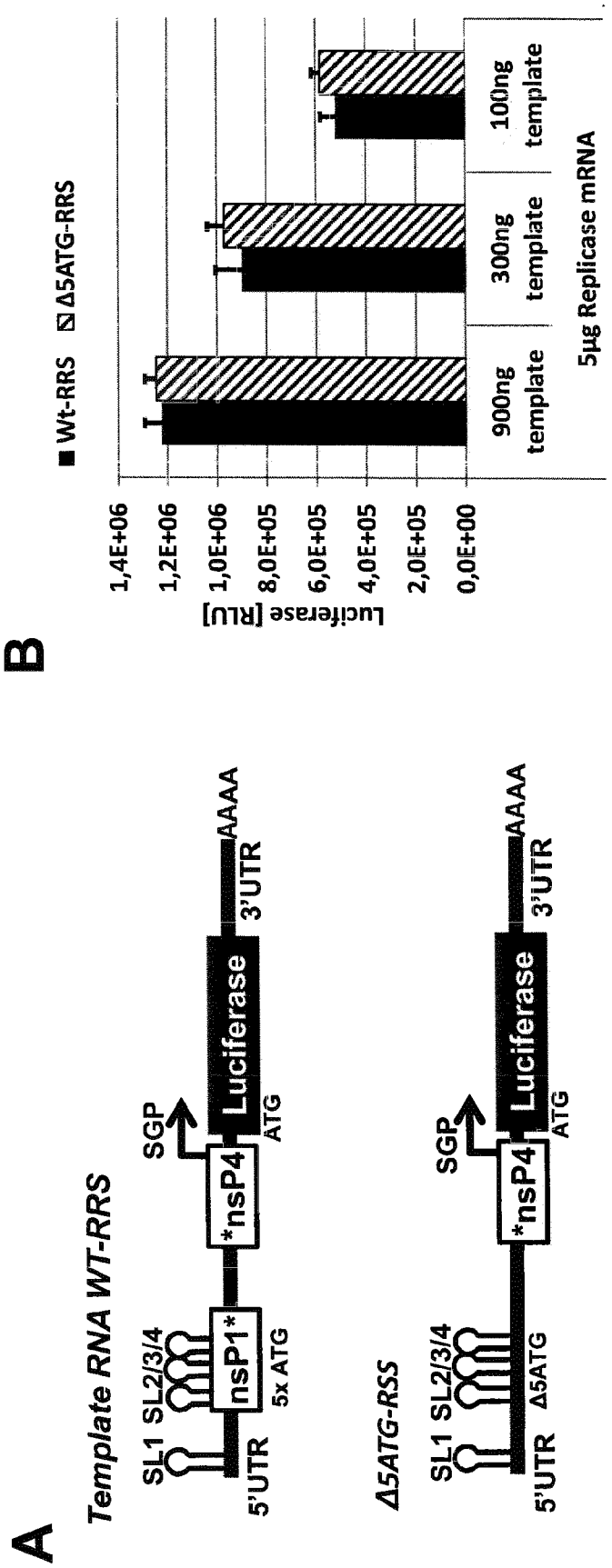

B
Wt-RRS
Δ5ATG-RRS
1,4E+06
1,2E+06
1,0E+06
8,0E+05
6,0E+05
4,0E+05
2,0E+05
0,0E+00
Luciferase [RLU]
900ng template
300ng template
100ng template
5µg Replicase mRNA
A
Template RNA WT-RRS
SL1
SL2/3/4
nsP1*
5x ATG
5'UTR
*nsP4
SGP
Luciferase
ATG
3'UTR
AAAA
Δ5ATG-RSS
Δ5ATG

Fig. 2

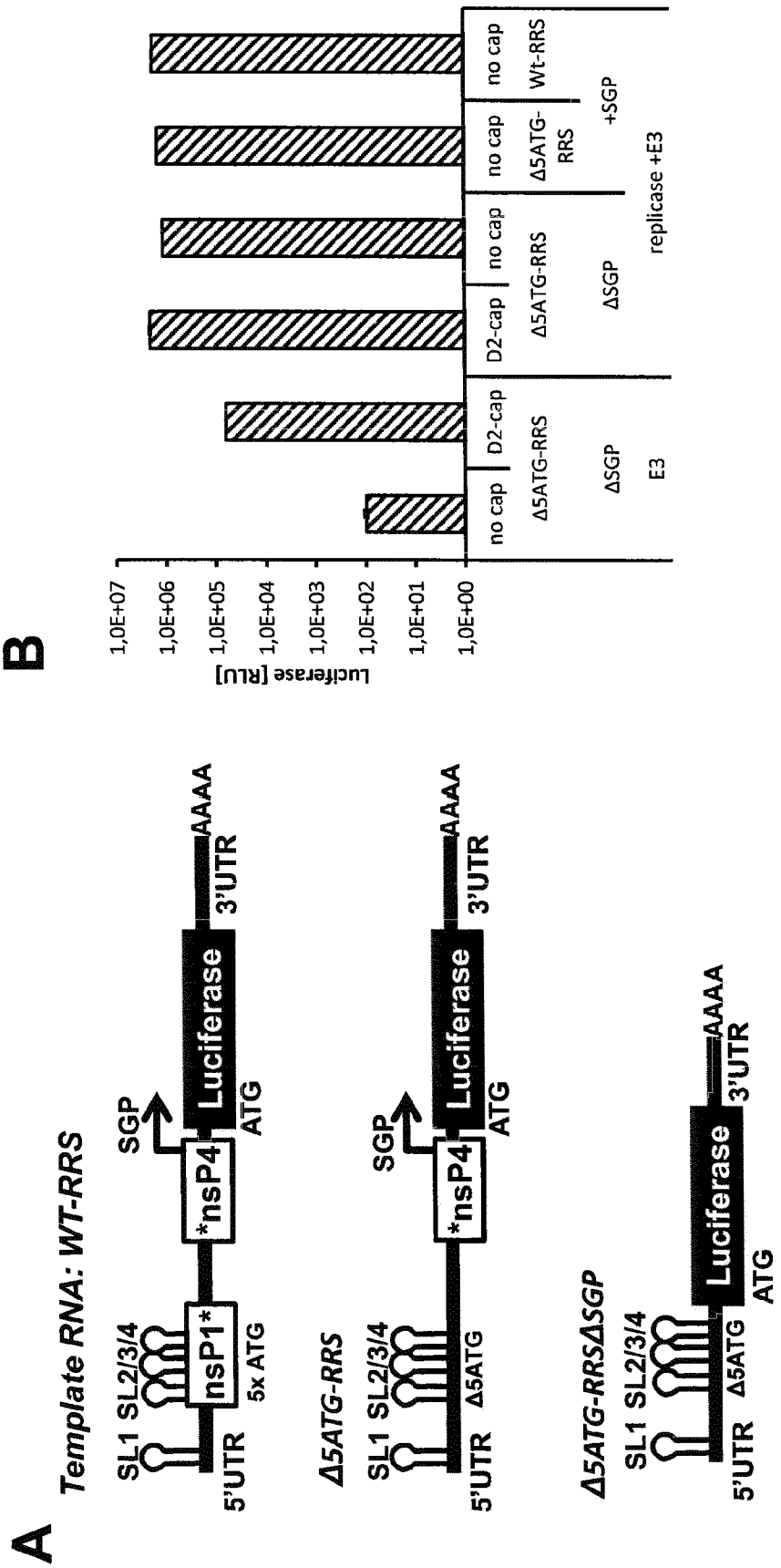

A
Template RNA: WT-RRS
SL1 SL2/3/4
5'UTR
nsP1*
5x ATG
*nsP4
SGP
Luciferase
ATG
3'UTR
AAAA
Δ5ATG-RRS
Δ5ATG
Δ5ATG-RRSΔSGP
B
Luciferase [RLU]
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
1,0E+00
no cap
D2-cap
Δ5ATG-RRS
ΔSGP
E3
Δ5ATG-RRS
ΔSGP
replicase +E3
Δ5ATG-RRS
+SGP
Wt-RRS

Fig. 3

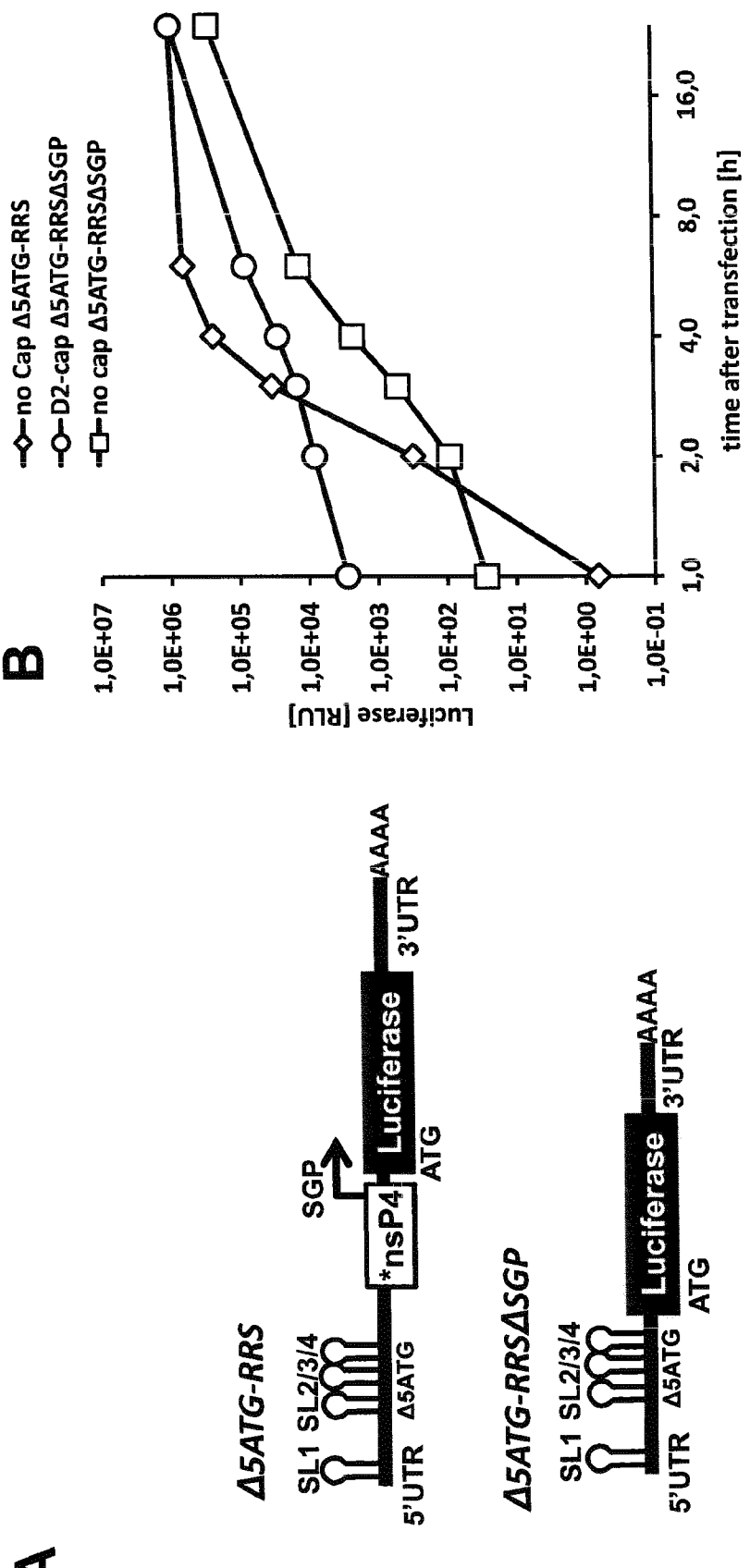

A
Δ5ATG-RRS
SL1 SL2/3/4
5'UTR
Δ5ATG
*nsP4
SGP
Luciferase
ATG
3'UTR
AAAA
Δ5ATG-RRSΔSGP
SL1 SL2/3/4
5'UTR
Δ5ATG
Luciferase
ATG
3'UTR
AAAA
B
no Cap Δ5ATG-RRS
D2-cap Δ5ATG-RRSΔSGP
no cap Δ5ATG-RRSΔSGP
Luciferase [RLU]
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
1,0E+00
1,0E-01
1,0
2,0
4,0
8,0
16,0
time after transfection [h]

Fig. 4

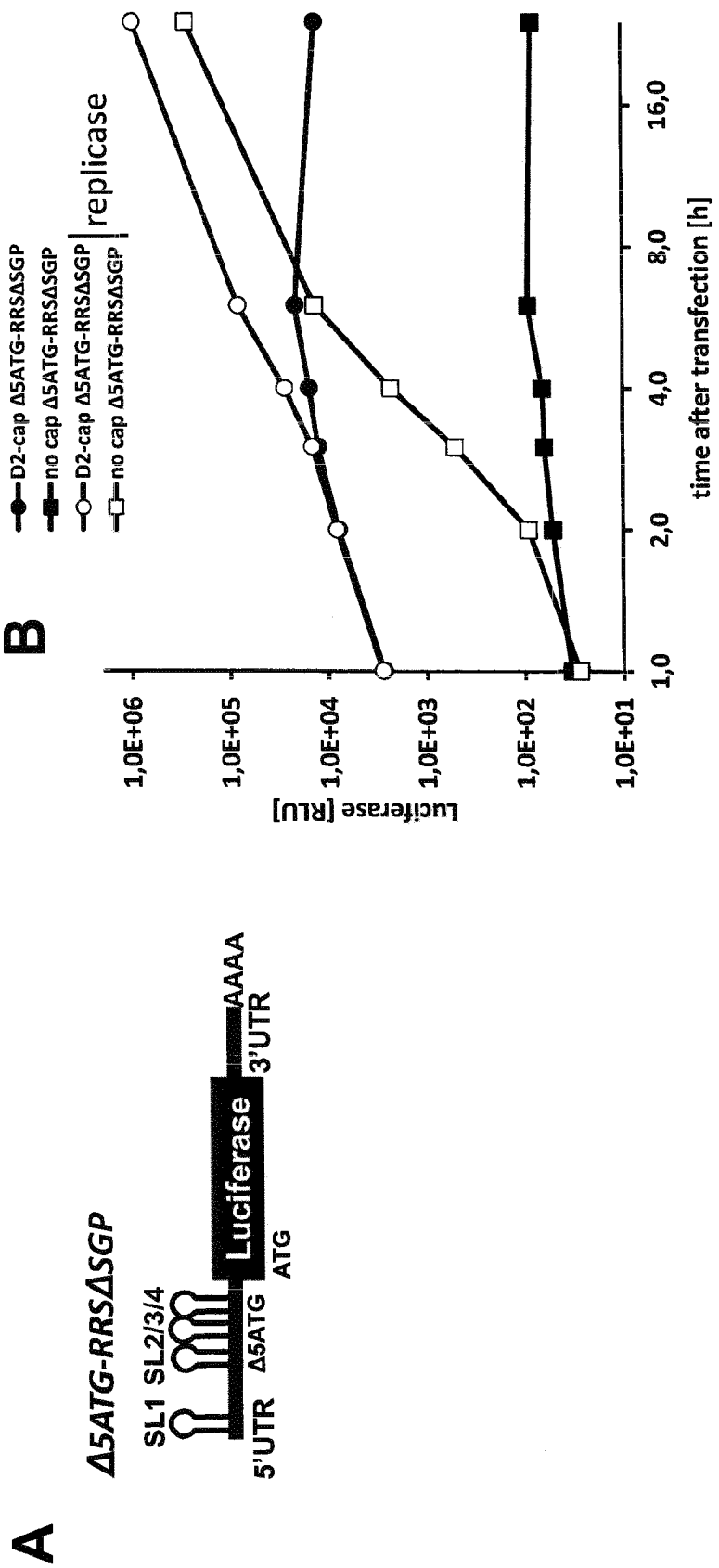

A
Δ5ATG-RRSΔSGP
SL1 SL2/3/4
5'UTR
Δ5ATG
Luciferase
ATG
3'UTR
AAAA
B
D2-cap Δ5ATG-RRSΔSGP
no cap Δ5ATG-RRSΔSGP
D2-cap Δ5ATG-RRSΔSGP
no cap Δ5ATG-RRSΔSGP
replicase
Luciferase [RLU]
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
1,0
2,0
4,0
8,0
16,0
time after transfection [h]

Fig. 5

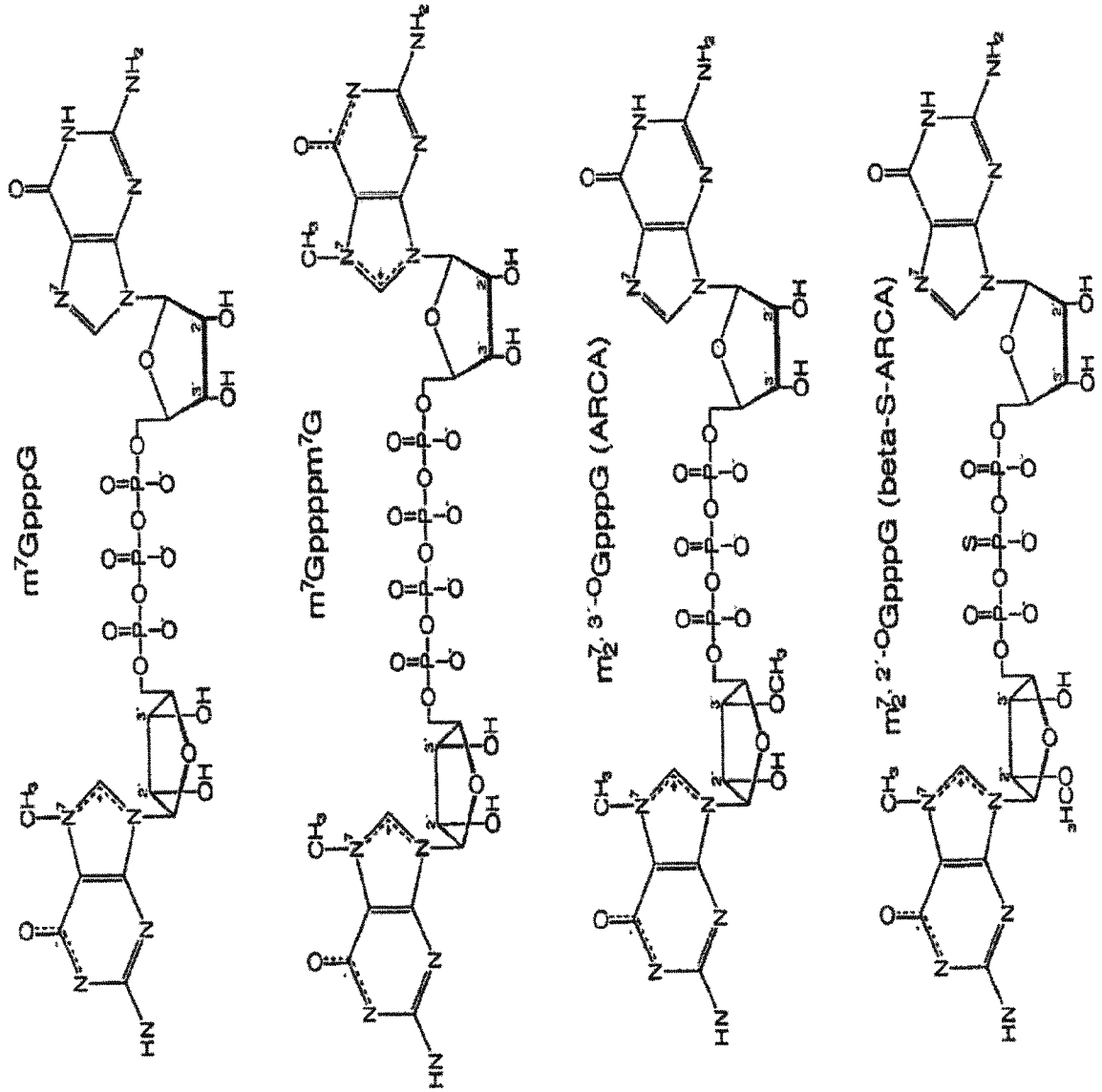


Fig. 6

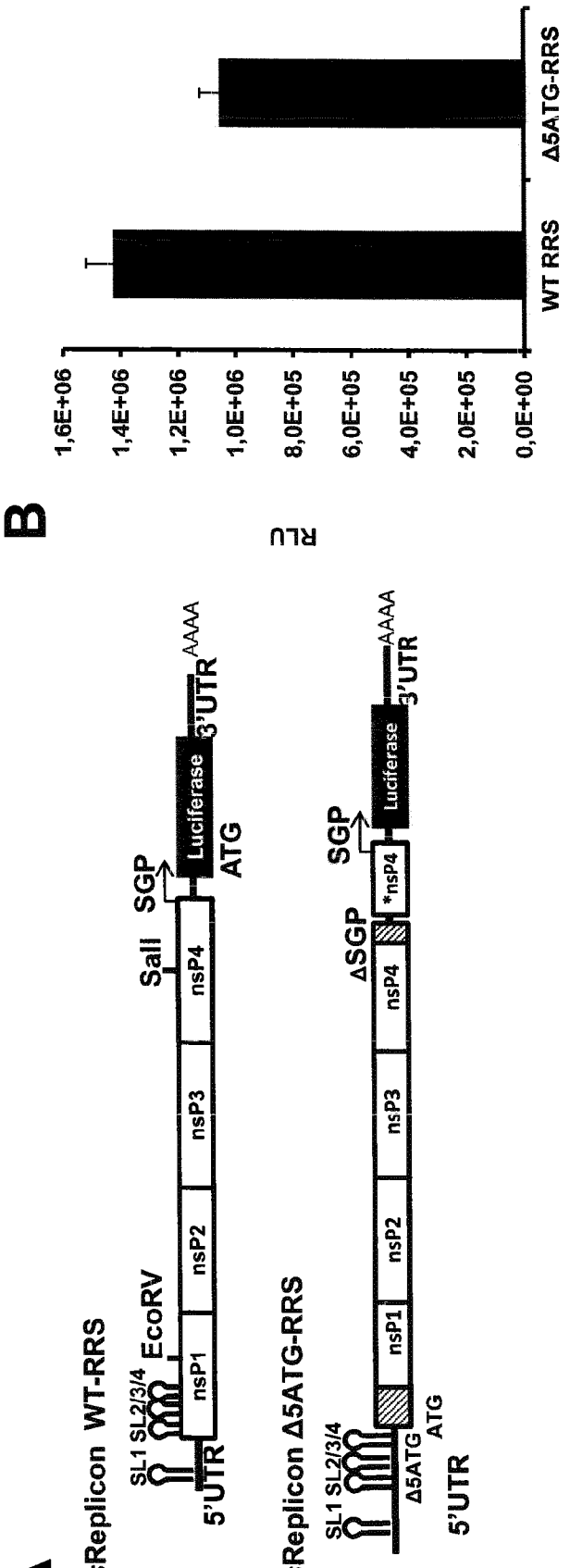
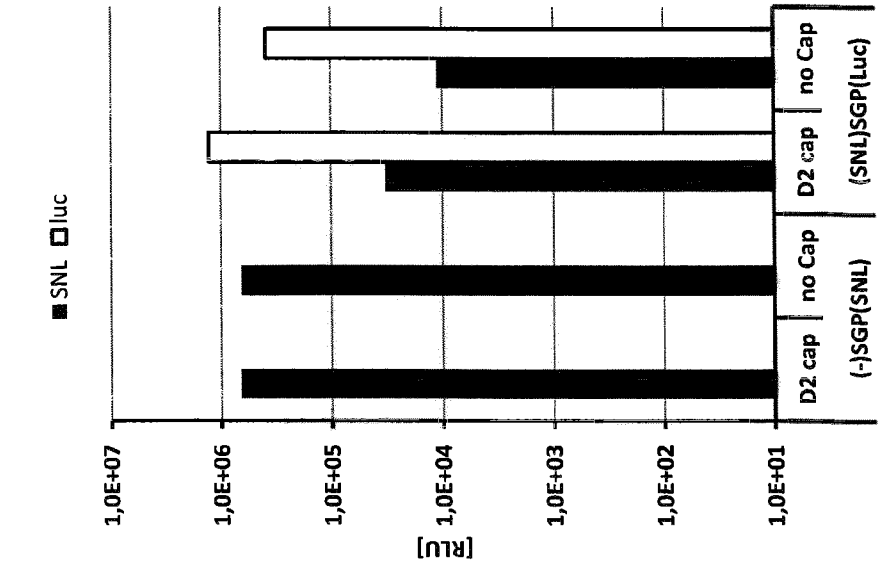

A
cisReplicon WT-RRS
5'UTR
SL1
SL2/3/4
EcoRV
nsP1
nsP2
nsP3
nsP4
SalI
SGP
Luciferase
ATG
3'UTR
AAAA
cisReplicon Δ5ATG-RRS
Δ5ATG
ATG
ΔSGP
*nsP4
B
RLU
1,6E+06
1,4E+06
1,2E+06
1,0E+06
8,0E+05
6,0E+05
4,0E+05
2,0E+05
0,0E+00
WT RRS
Δ5ATG-RRS

Fig. 7


B
SNL luc
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
[RLU]
D2 cap
no Cap
(-)SGP(SNL)
D2 cap
no Cap
(SNL)SGP(Luc)

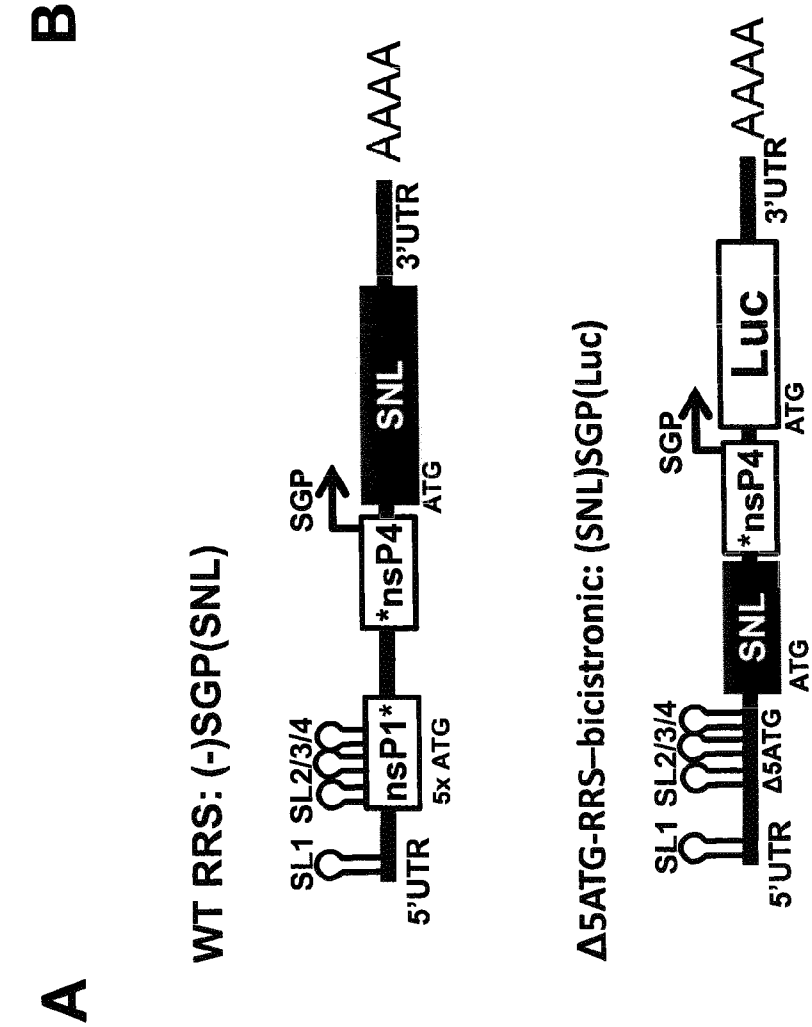

A
WT RRS: (-)SGP(SNL)
SL1
SL2/3/4
5'UTR
nsP1*
5x ATG
*nsP4
SGP
SNL
ATG
3'UTR
AAAA
Δ5ATG-RRS–bicistronic: (SNL)SGP(Luc)
SL1
SL2/3/4
5'UTR
Δ5ATG
SNL
ATG
*nsP4
SGP
Luc
ATG
3'UTR
AAAA

Fig. 8

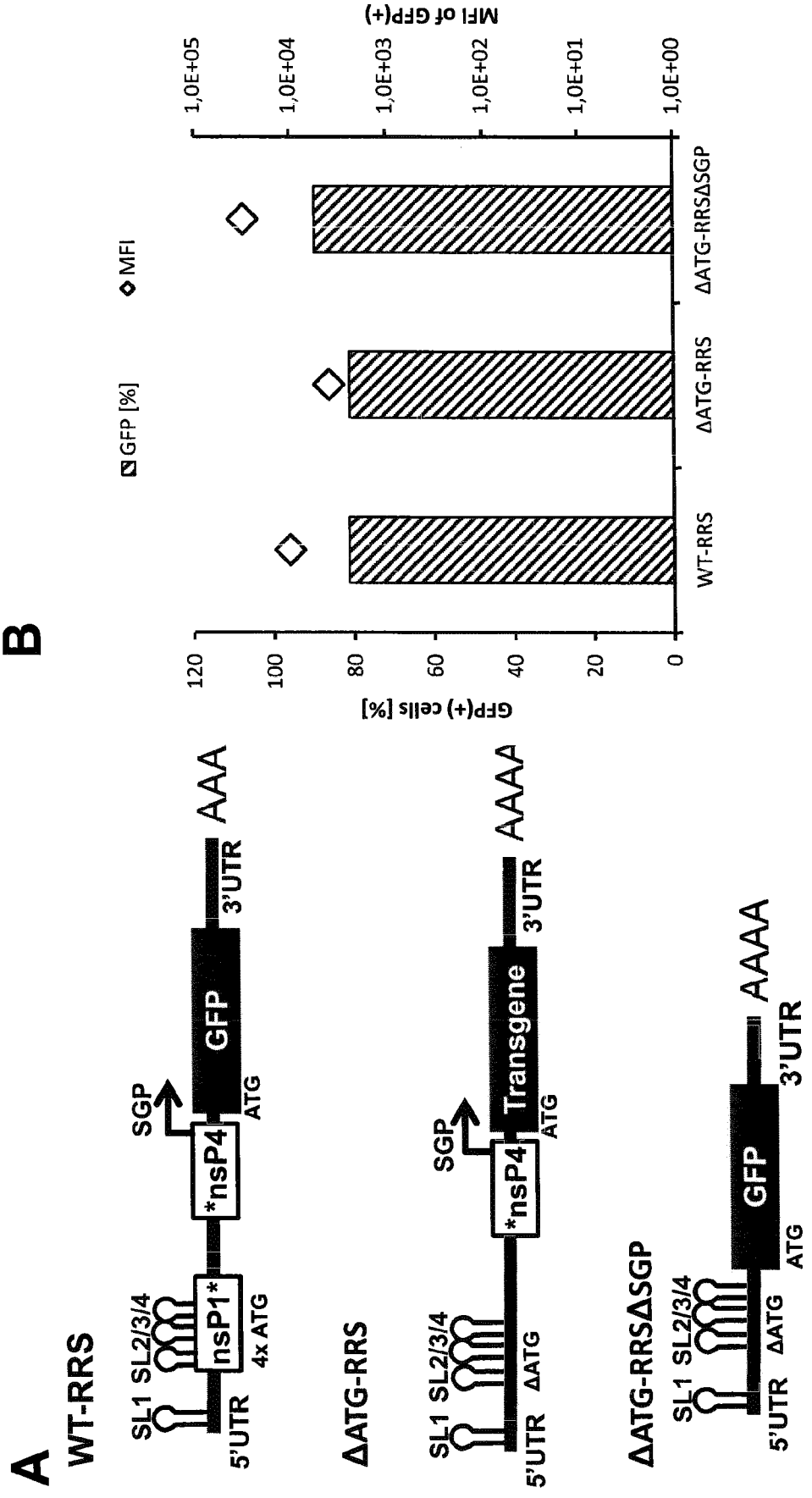

A
WT-RRS
5'UTR
SL1
SL2/3/4
nsP1*
4x ATG
*nsP4
SGP
GFP
ATG
3'UTR
AAA
ΔATG-RRS
5'UTR
SL1
SL2/3/4
ΔATG
*nsP4
SGP
Transgene
ATG
3'UTR
AAAA
ΔATG-RRSΔSGP
5'UTR
SL1
SL2/3/4
ΔATG
GFP
ATG
3'UTR
AAAA
B
GFP(+) cells [%]
120
100
80
60
40
20
0
MFI of GFP(+)
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
1,0E+00
GFP [%]
MFI
WT-RRS
ΔATG-RRS
ΔATG-RRSΔSGP

Fig. 9