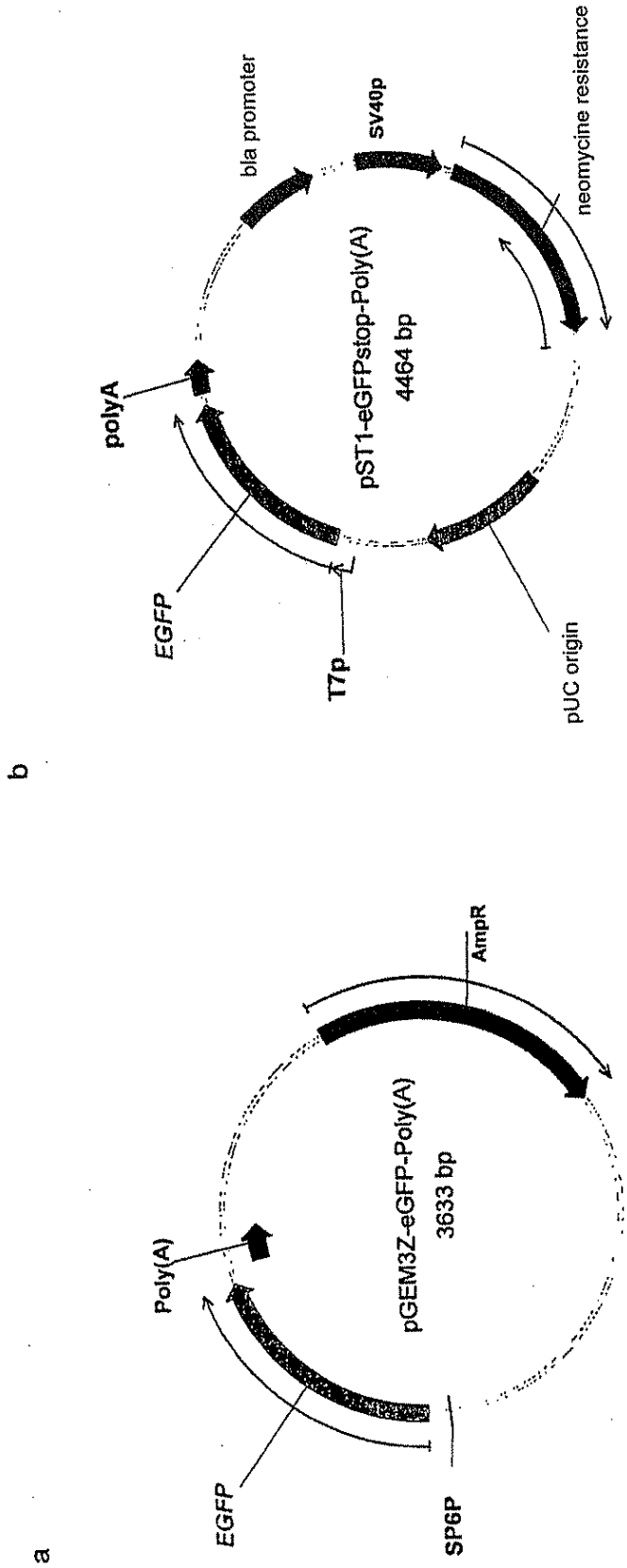
b
bla promoter
SV40p
neomycine resistance
polyA
pST1-eGFPstop-Poly(A)
4464 bp
EGFP
T7p
pUC origin
a
AmpR
Poly(A)
pGEM3Z-eGFP-Poly(A)
3633 bp
EGFP
SP6P

Fig. 1

Fig. 1

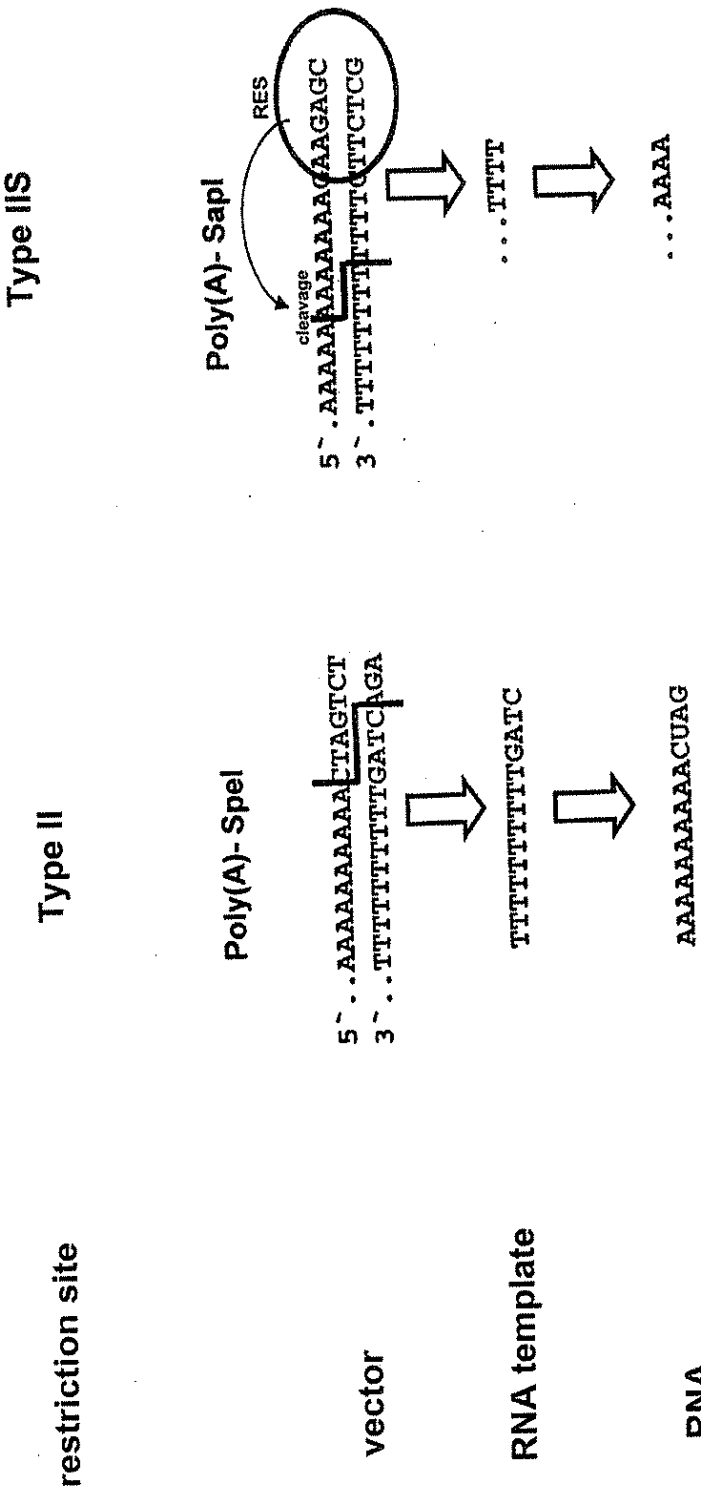
Type IIS
Poly(A)- SapI
RES
cleavage
5`. AAAAAAAAAAAAAAAAGAAGAGC
3`. TTTTTTTTTTTTTTTTCTTCTCG
. . .TTTT
. . .AAAA
Type II
Poly(A)- SpeI
5`. . AAAAAAAAAACTAGTCT
3`. . TTTTTTTTTTGATCAGA
TTTTTTTTTTGATC
AAAAAAAAAACUAG
restriction site
vector
RNA template
RNA

Fig. 2

Fig. 3

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vector constructs
IVT vectors
mRNA transcripts
c
pGEM3Z-eGFP-A120-SapI
Cap eGFP A(120)3`
pGEM3Z-eGFP- 1BgUTR -A120-SapI
Cap eGFP 3'βg A(120)3`
pGEM3Z-eGFP- 2BgUTR -A120-SapI
Cap eGFP 3'βg 3'βg A(120)3`
d
pST1-Sec-SIINFEKL- 2BgUTR -A120-SapI
Cap sec SIINFEKL 3'βg 3'βg A(120)3`
pST1-Sec-SIINFEKL- 2BgUTR -A120-SpeI
Cap sec SIINFEKL 3'βg 3'βg A(120)CTAGT3`
pST1-eGFP-A67-SpeI
Cap sec SIINFEKL 3'βg 3'βg A(67)CTAGT3`
pST1-Sec-SIINFEKL- A67-SpeI
Cap sec SIINFEKL A(67)CTAGT3`
pST1-Sec-pp65- 2BgUTR -A120-SapI
Cap sec pp65 3'βg 3'βg A(120)3`
pST1-Sec-pp65-A67-SpeI
Cap sec pp65 A(67)CTAGT3`

Fig. 3

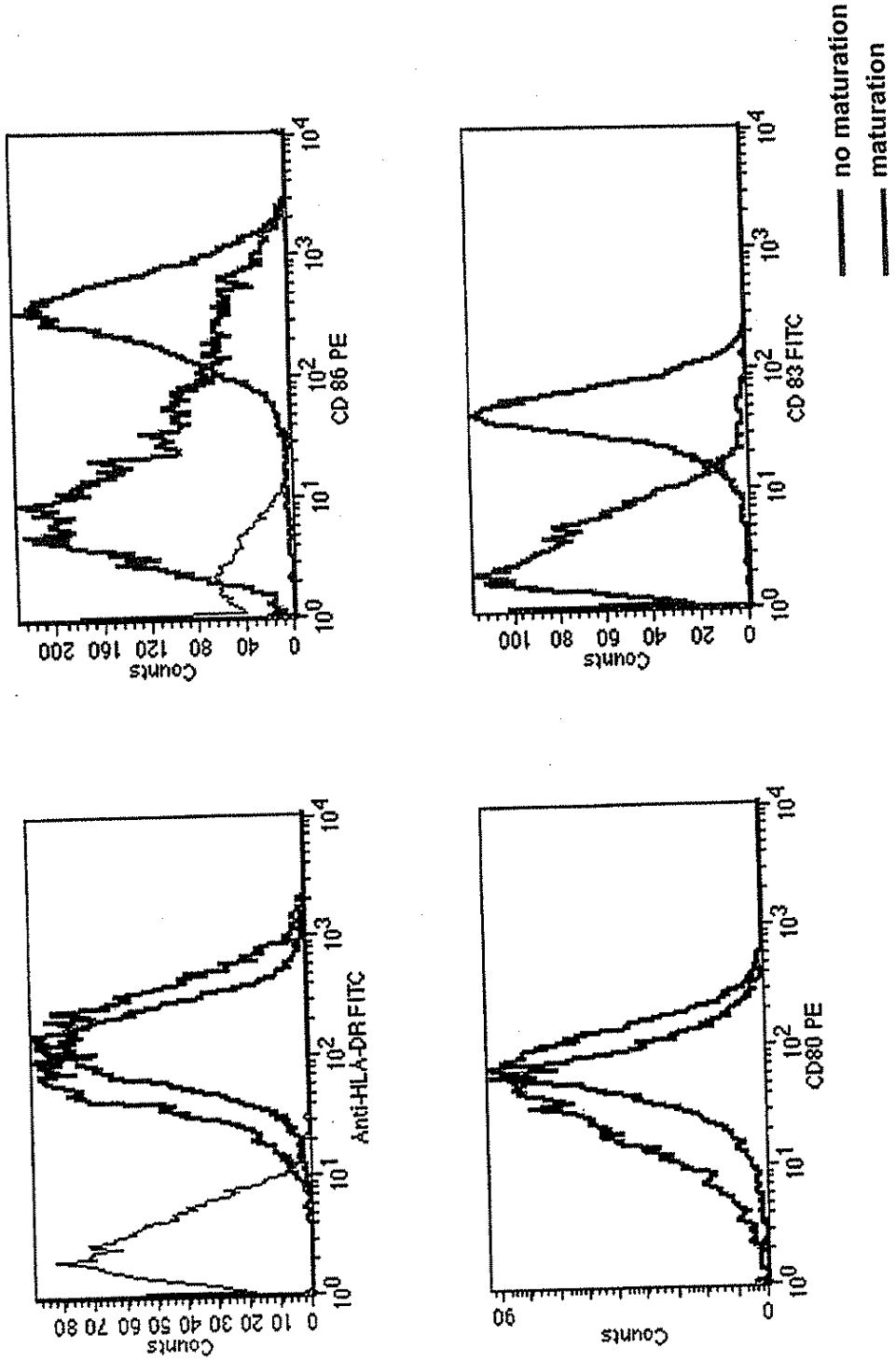
no maturation
maturation
CD 86 PE
CD 83 FITC
Anti-HLA-DR FITC
CD80 PE
Counts

Fig. 4

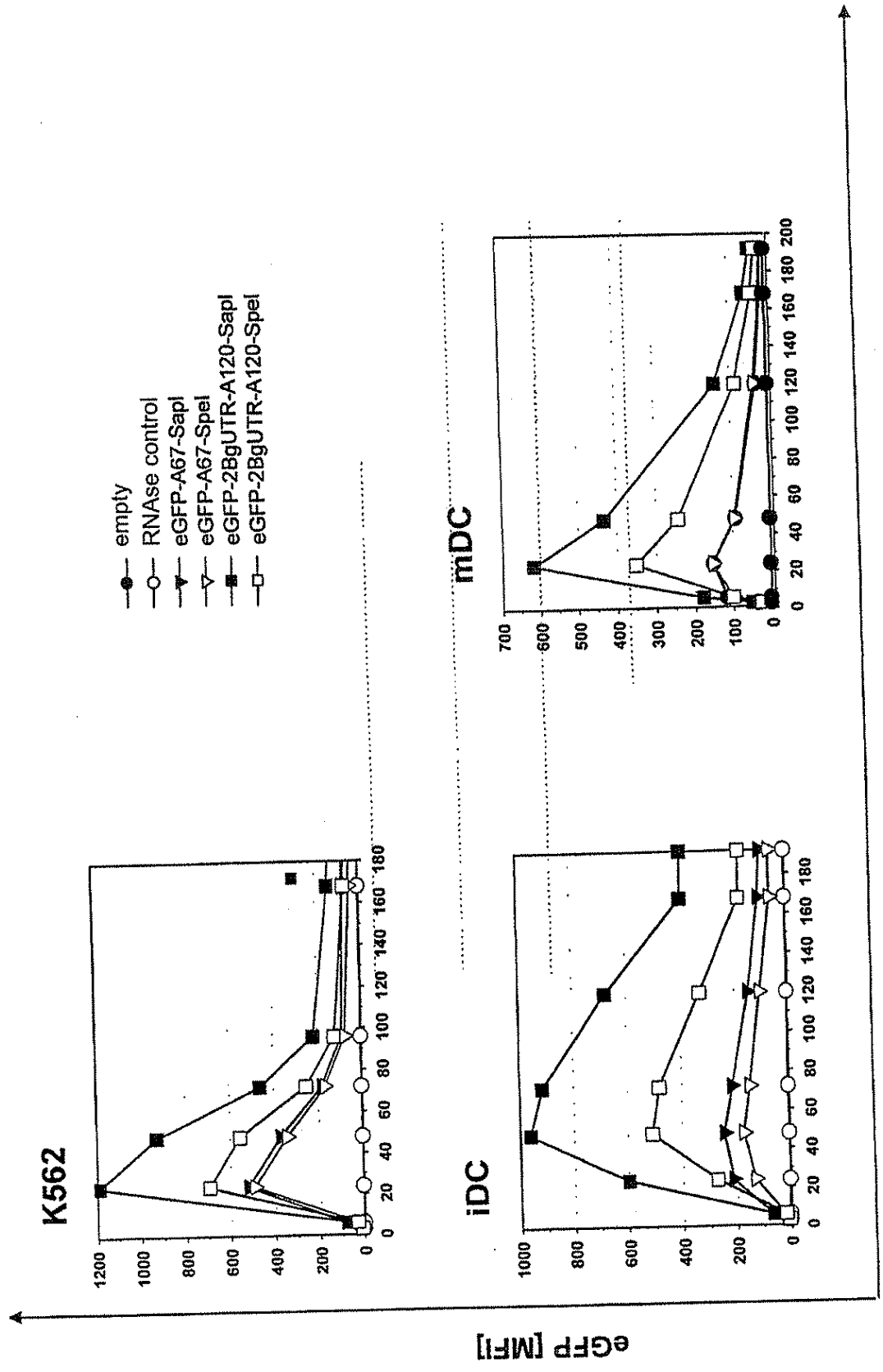
empty
RNAse control
eGFP-A67-SapI
eGFP-A67-SpeI
eGFP-2BgUTR-A120-SapI
eGFP-2BgUTR-A120-SpeI
K562
iDC
mDC
eGFP [MFI]
time [h]

Fig. 5(a)

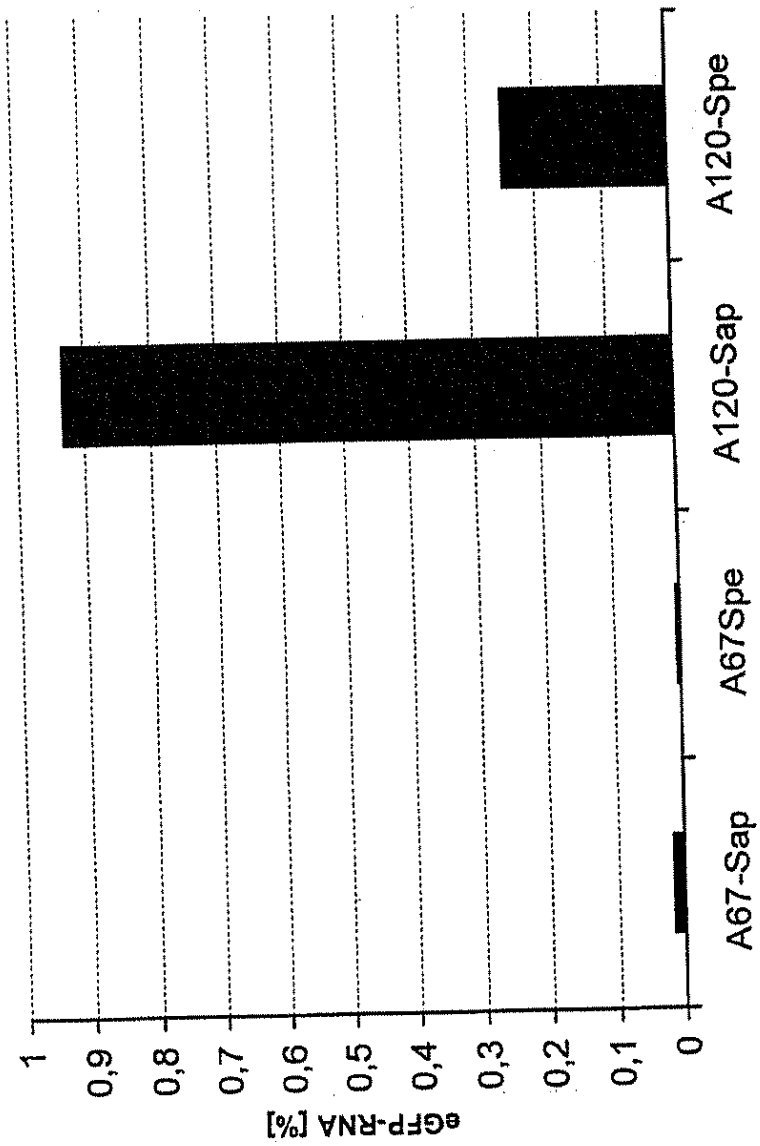

Fig. 5(b)

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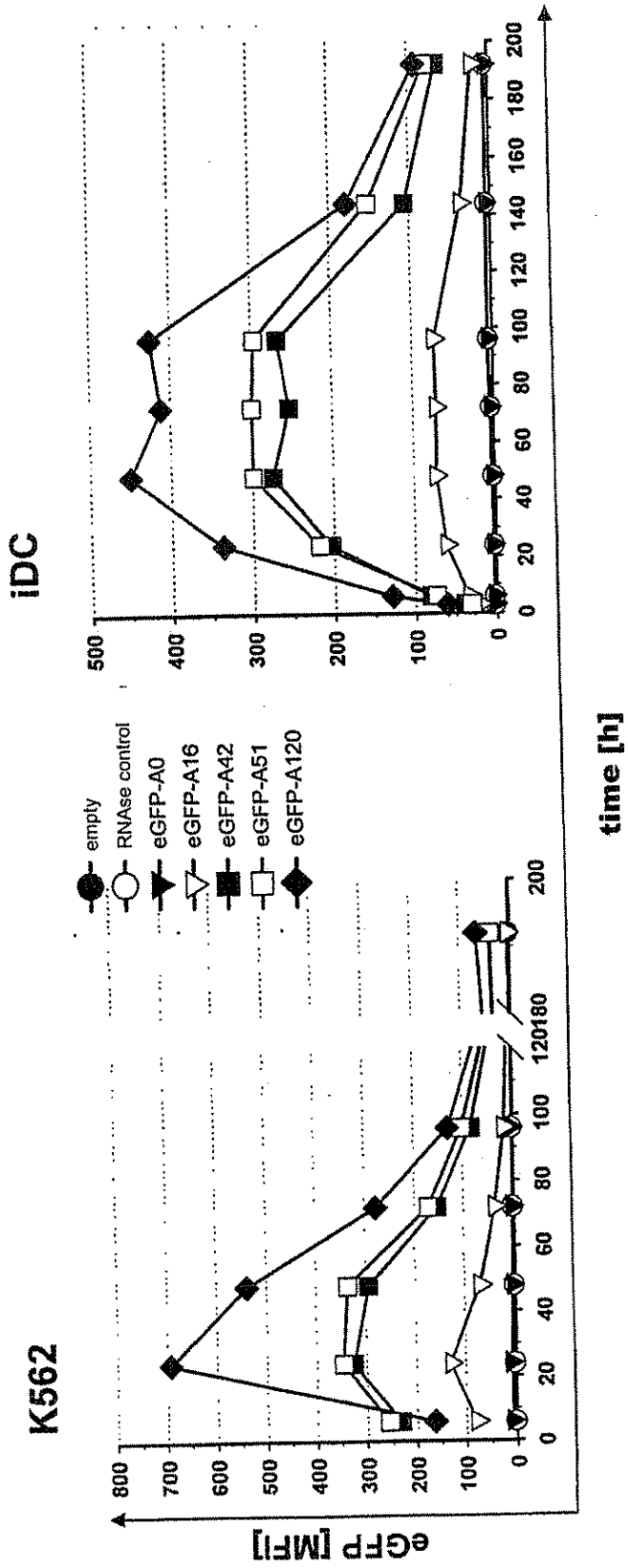

Fig. 6(a)

SHAKIRA N
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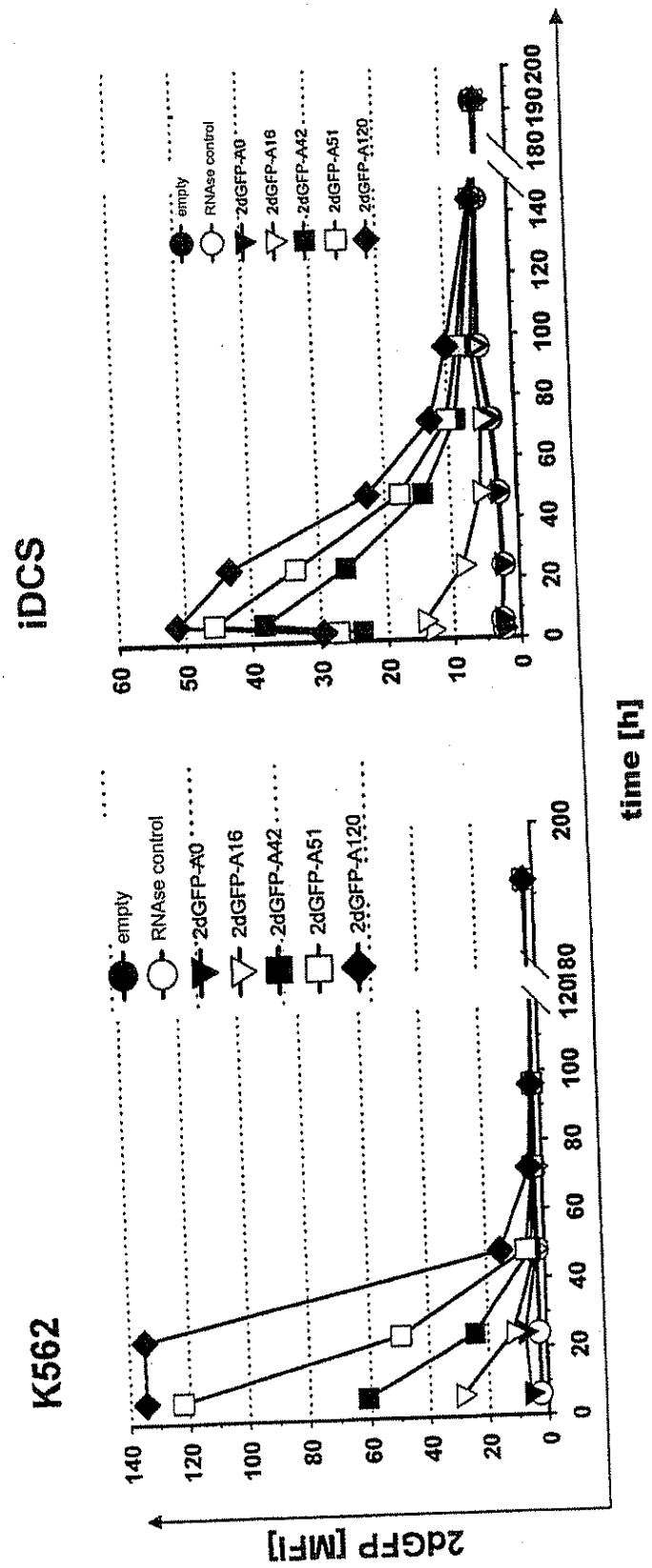
K562
iDCS
2dGFP [MFI]
time [h]
empty
RNAse control
2dGFP-A0
2dGFP-A16
2dGFP-A42
2dGFP-A51
2dGFP-A120

Fig. 6(b)

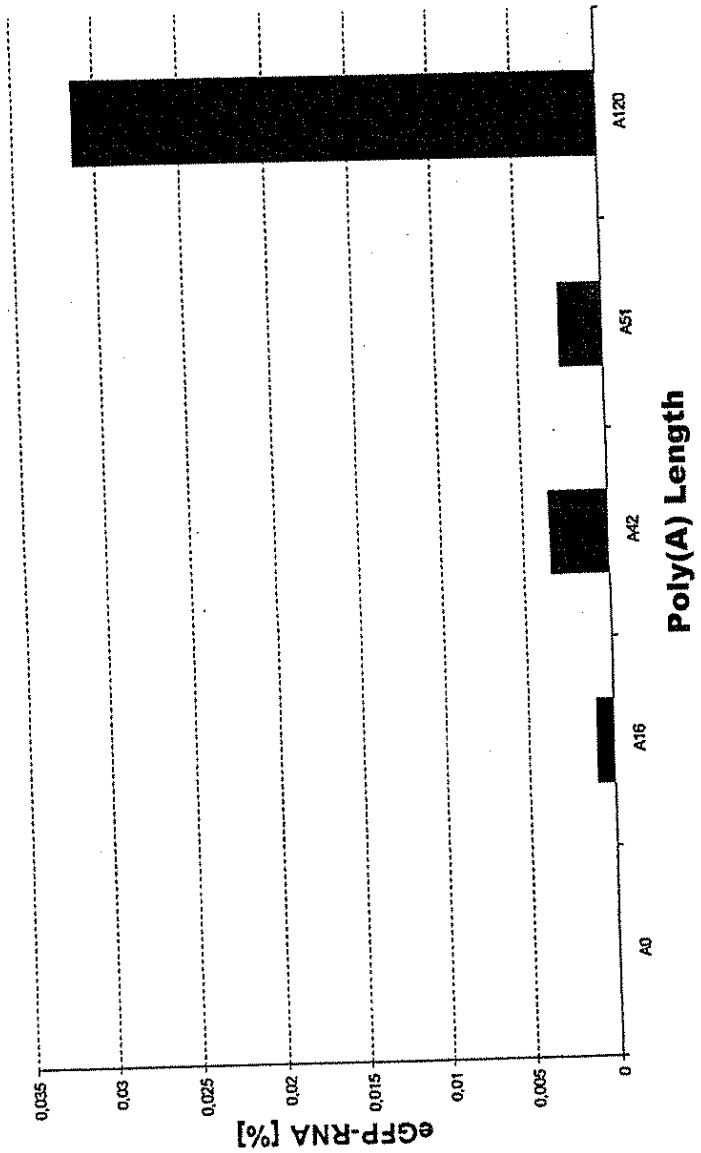

Fig. 6(c)

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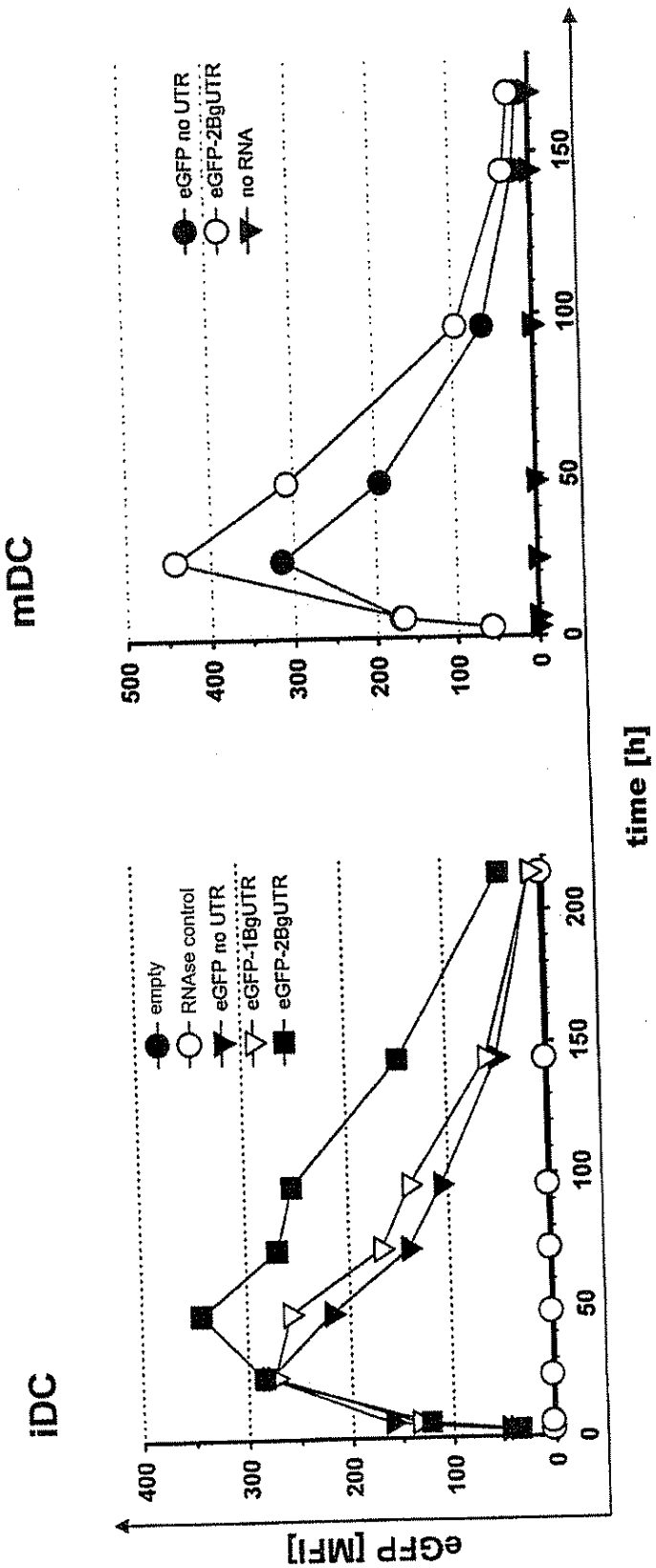

Fig. 7

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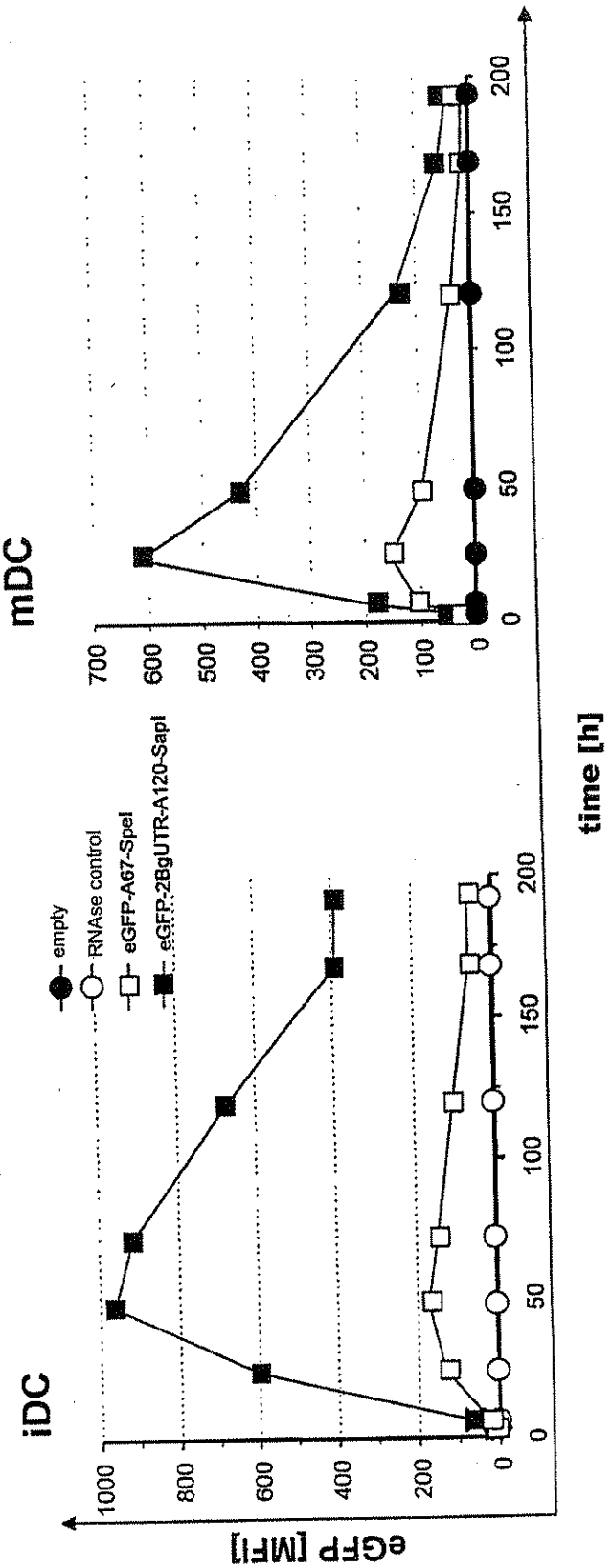

Fig. 8

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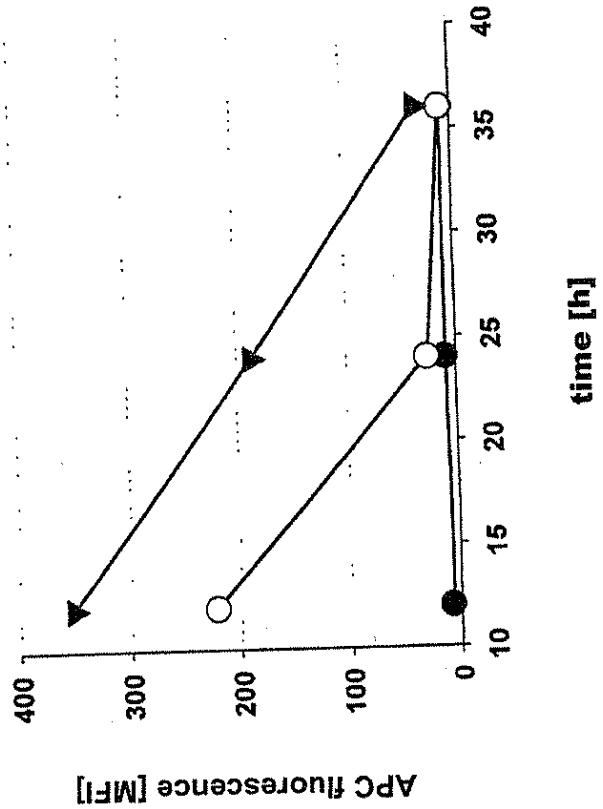

Fig. 9

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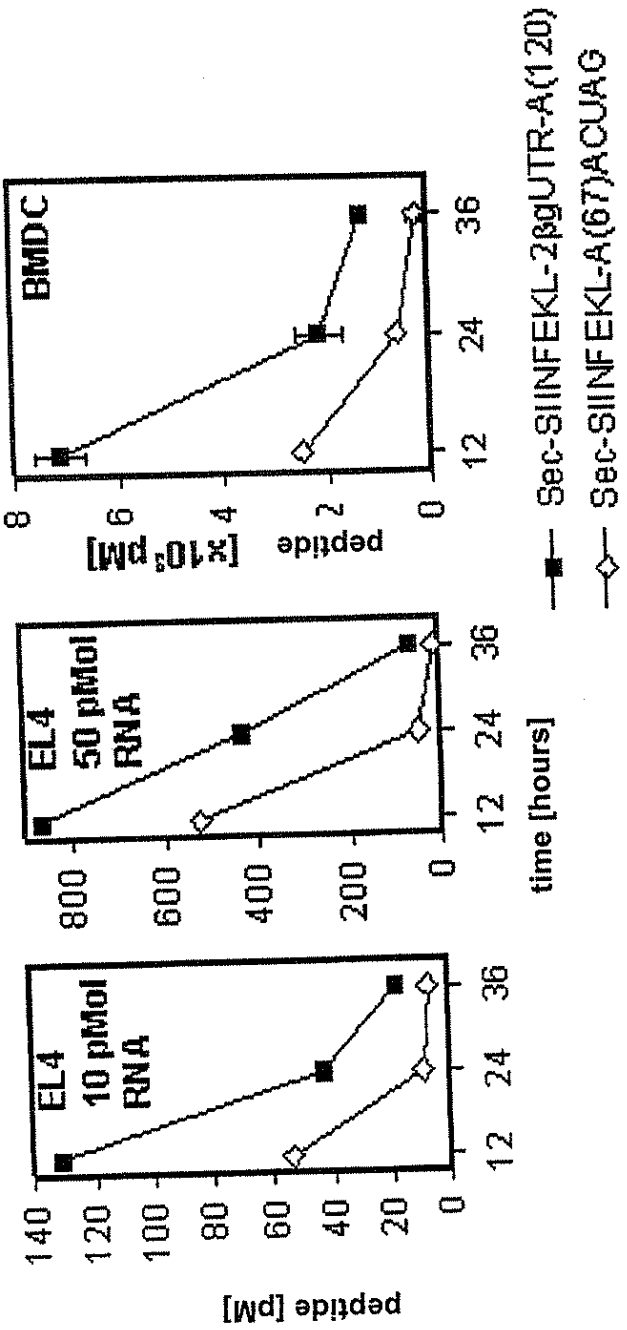

Fig. 10

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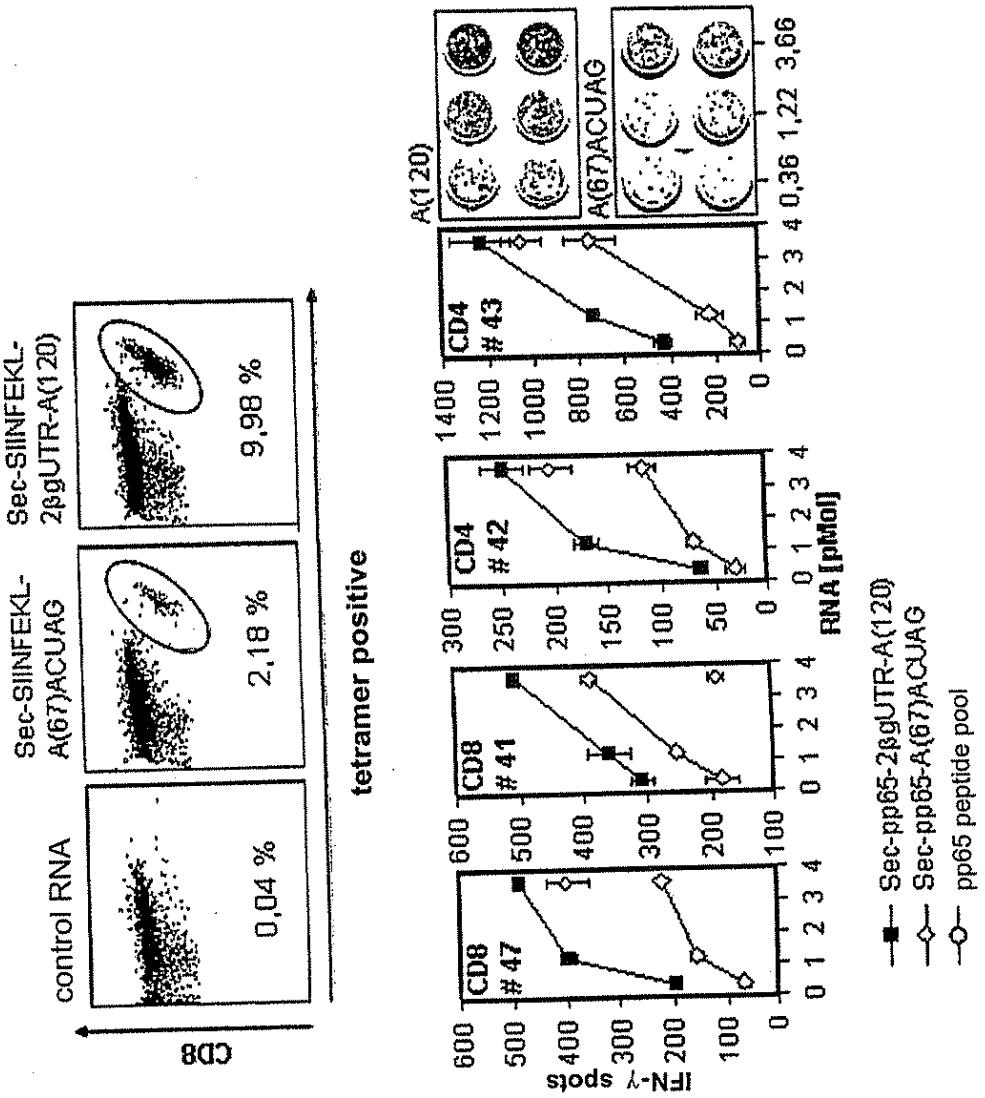

Fig. 11

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