FIG. 1

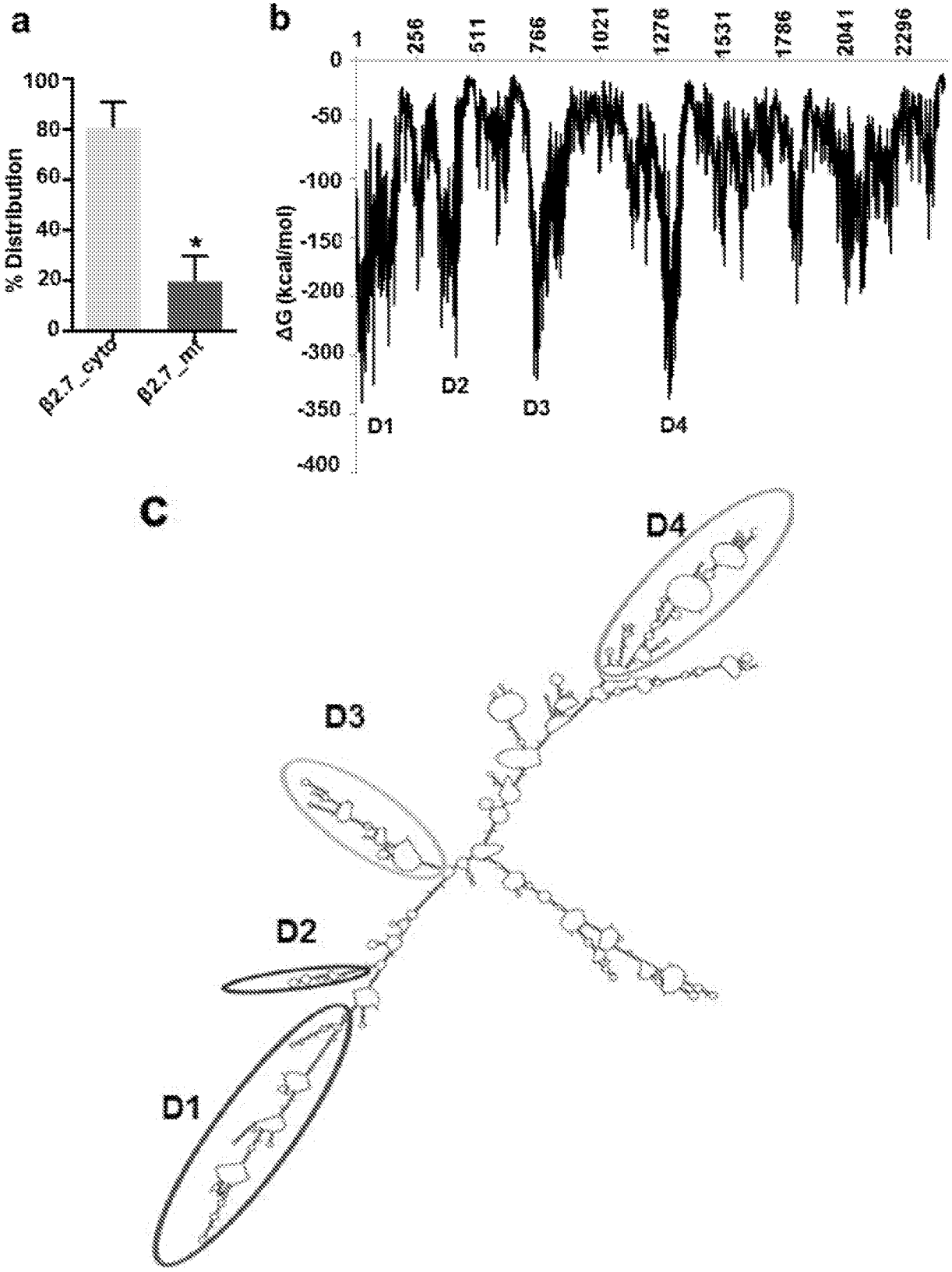

a
% Distribution
100
80
60
40
20
0
*
β2.7_cyto
β2.7_mt
b
ΔG (kcal/mol)
0
-50
-100
-150
-200
-300
-350
-400
1
256
511
766
1021
1276
1531
1786
2041
2296
D1
D2
D3
D4
C
D4
D3
D2
D1

FIG. 1 continued

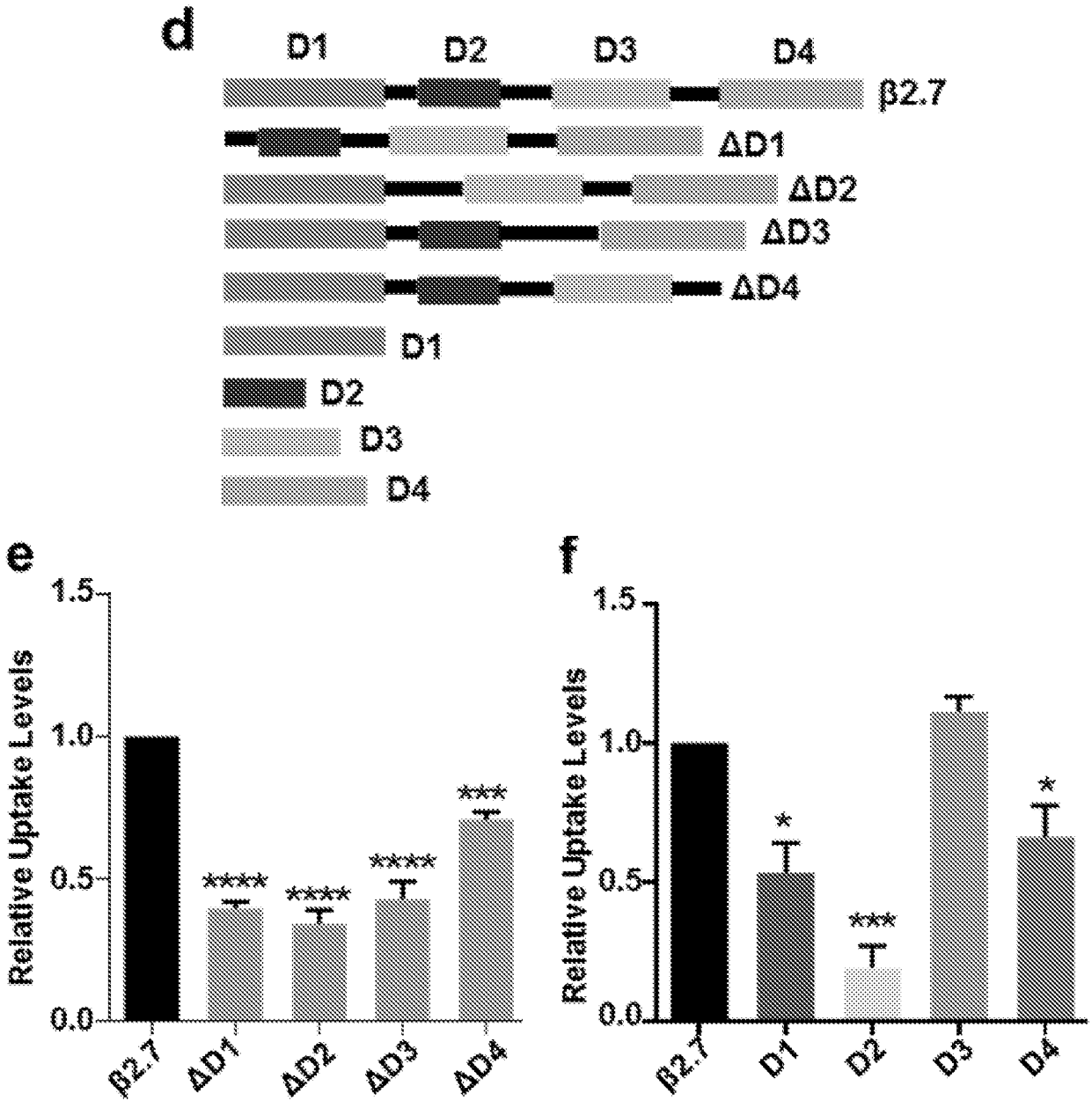


FIG. 2

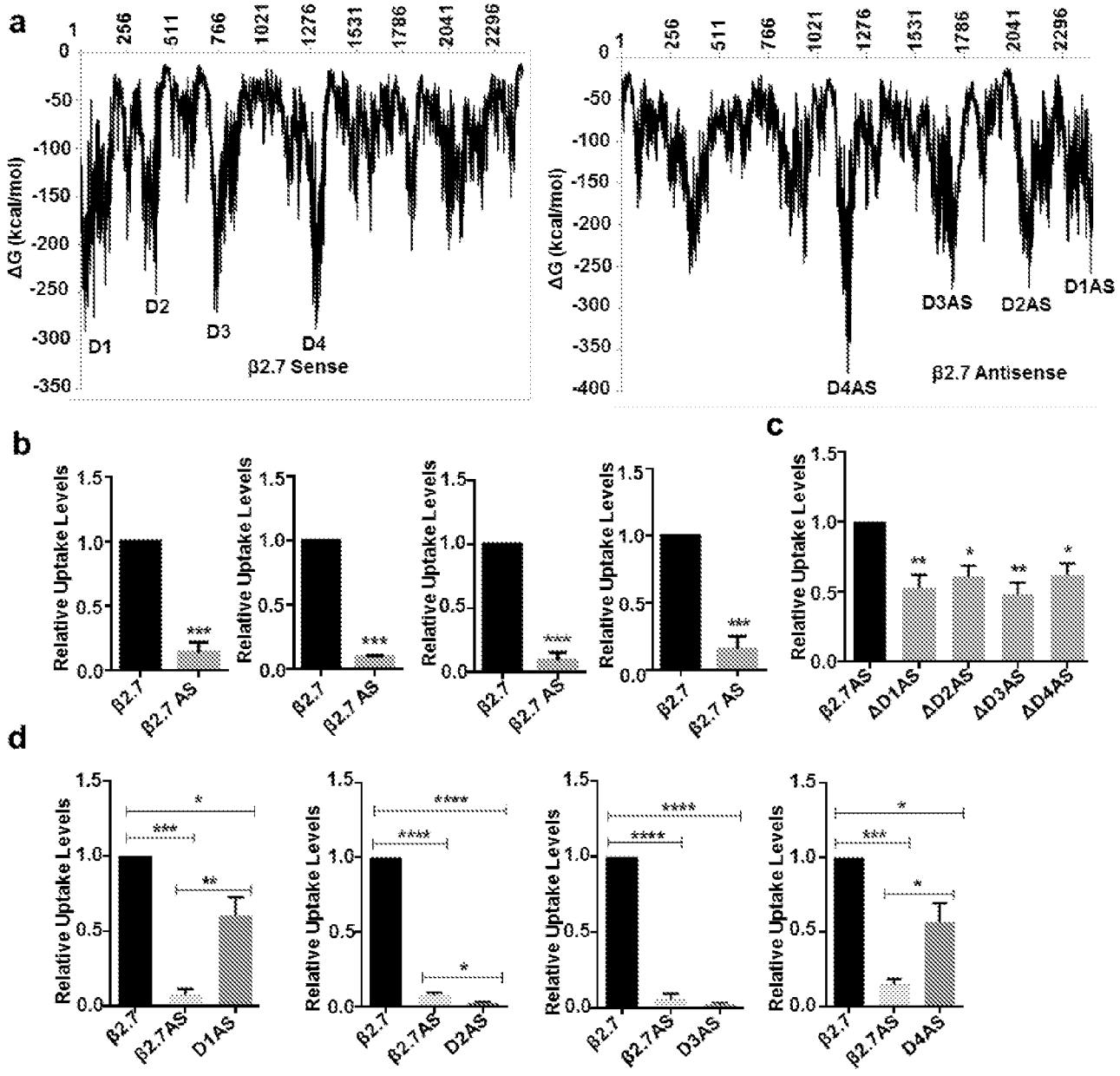

a
ΔG (kcal/mol)
D1
D2
D3
D4
β2.7 Sense
D4AS
D3AS
D2AS
D1AS
β2.7 Antisense
b
Relative Uptake Levels
β2.7
β2.7 AS
c
β2.7AS
ΔD1AS
ΔD2AS
ΔD3AS
ΔD4AS
d
D1AS
D2AS
D3AS
D4AS

FIG. 3

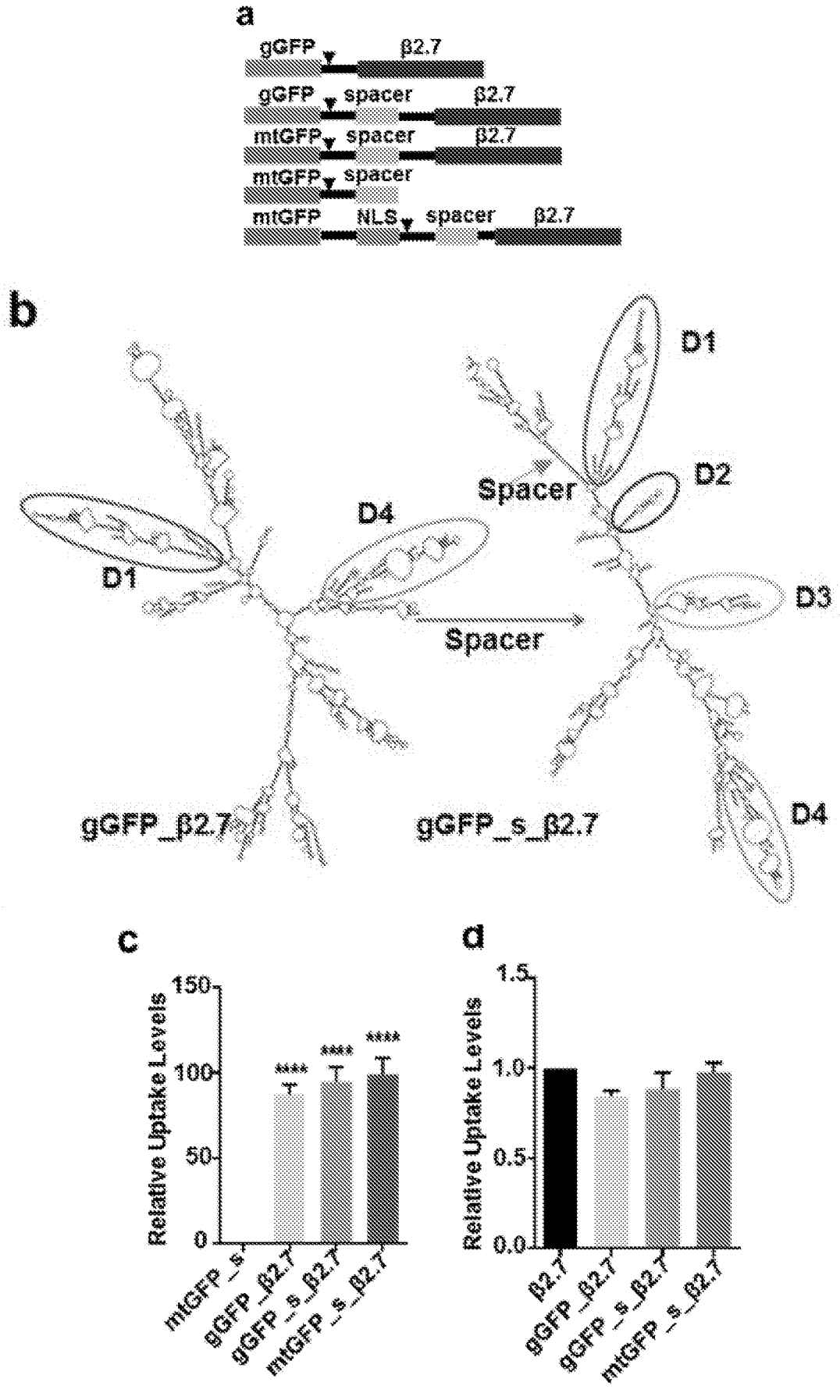

a
gGFP
β2.7
gGFP
spacer
β2.7
mtGFP
spacer
β2.7
mtGFP
spacer
mtGFP
NLS
spacer
β2.7
b
D1
D2
Spacer
D4
D1
D3
Spacer
gGFP_β2.7
gGFP_s_β2.7
D4
c
Relative Uptake Levels
150
100
50
0
****
****
****
mtGFP _s
gGFP _β2.7
gGFP _s_β2.7
mtGFP _s_β2.7
d
Relative Uptake Levels
1.5
1.0
0.5
0.0
β2.7
gGFP _β2.7
gGFP _s_β2.7
mtGFP _s_β2.7

FIG. 3 continued

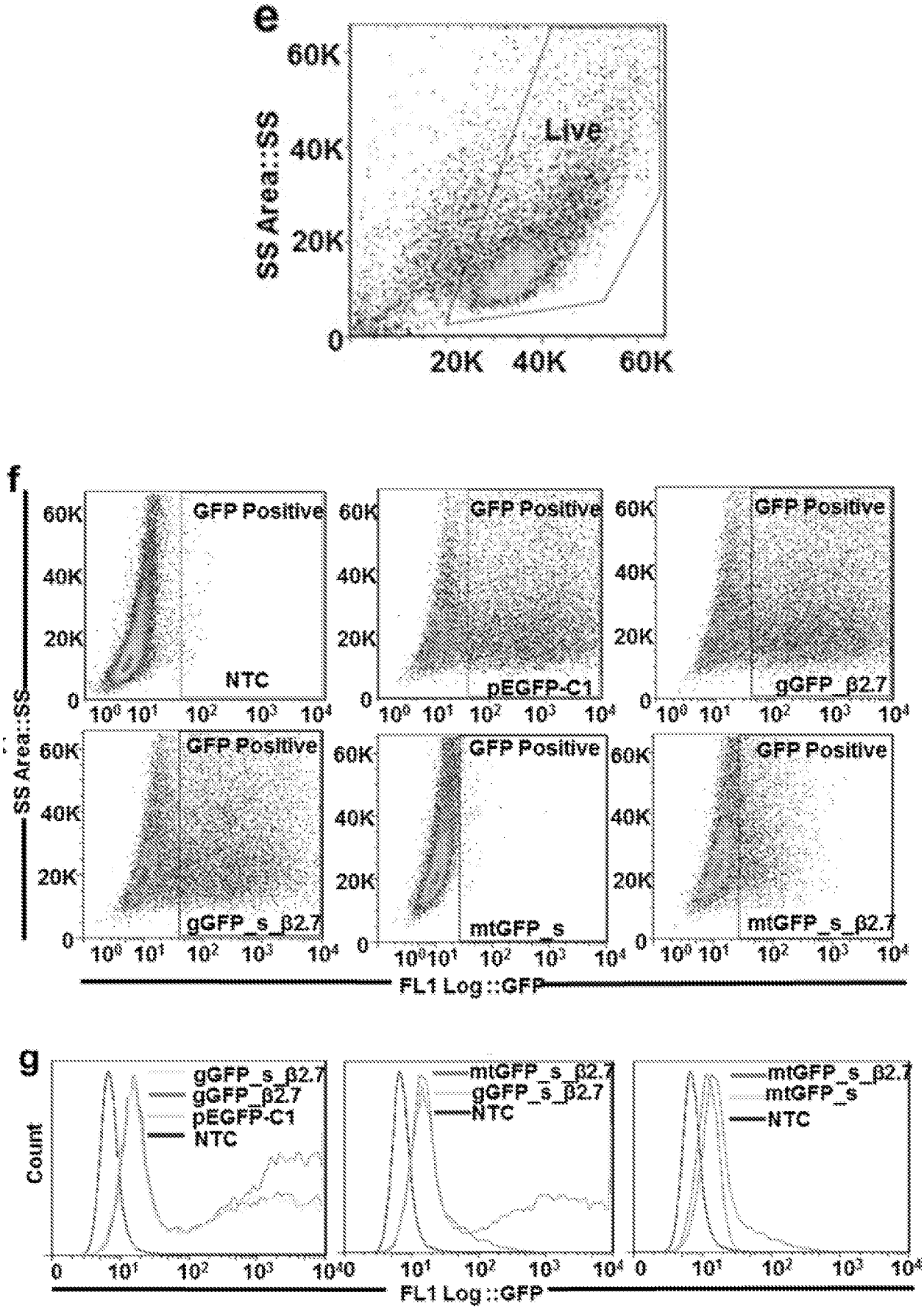


FIG. 3 continued

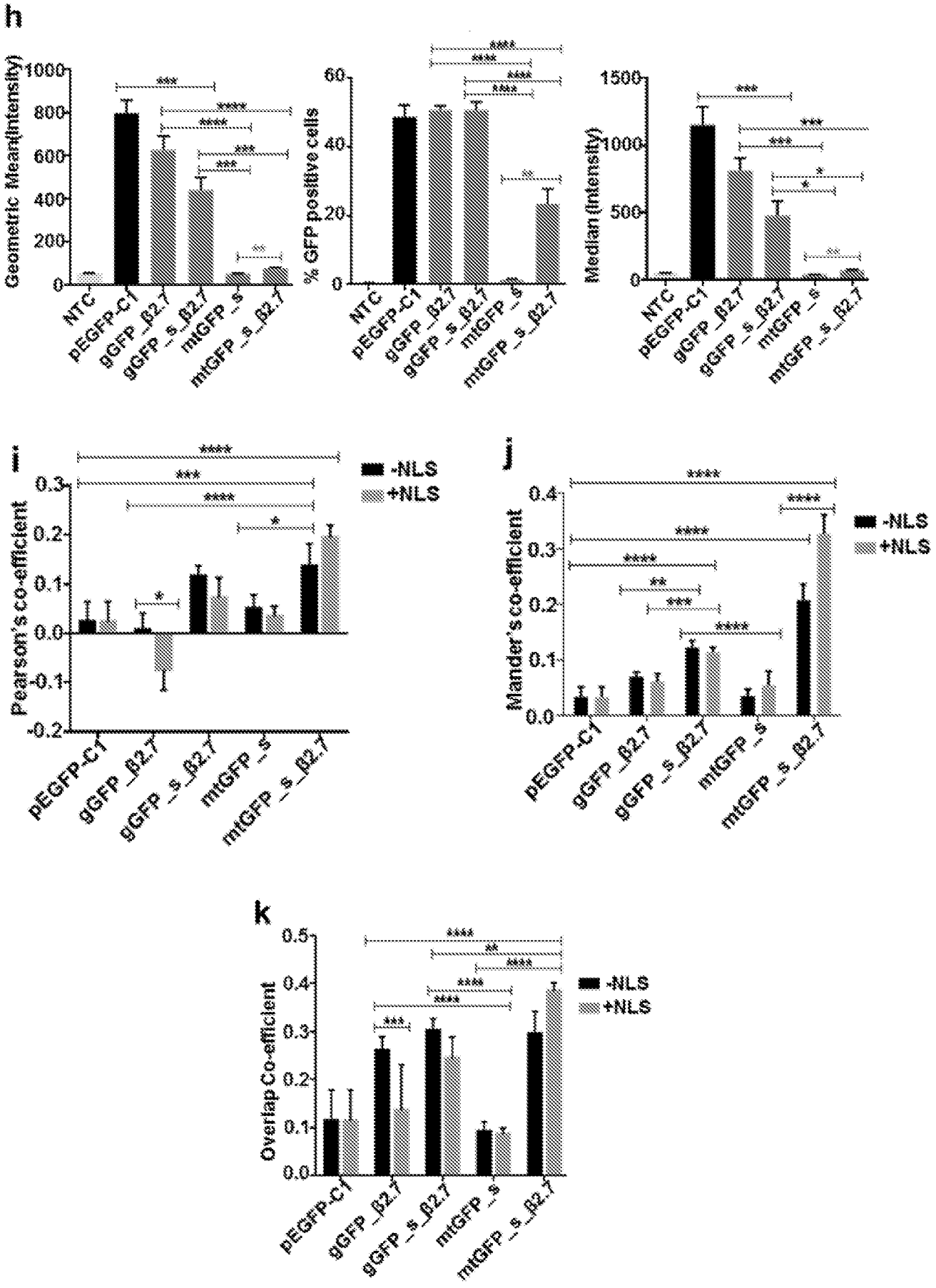
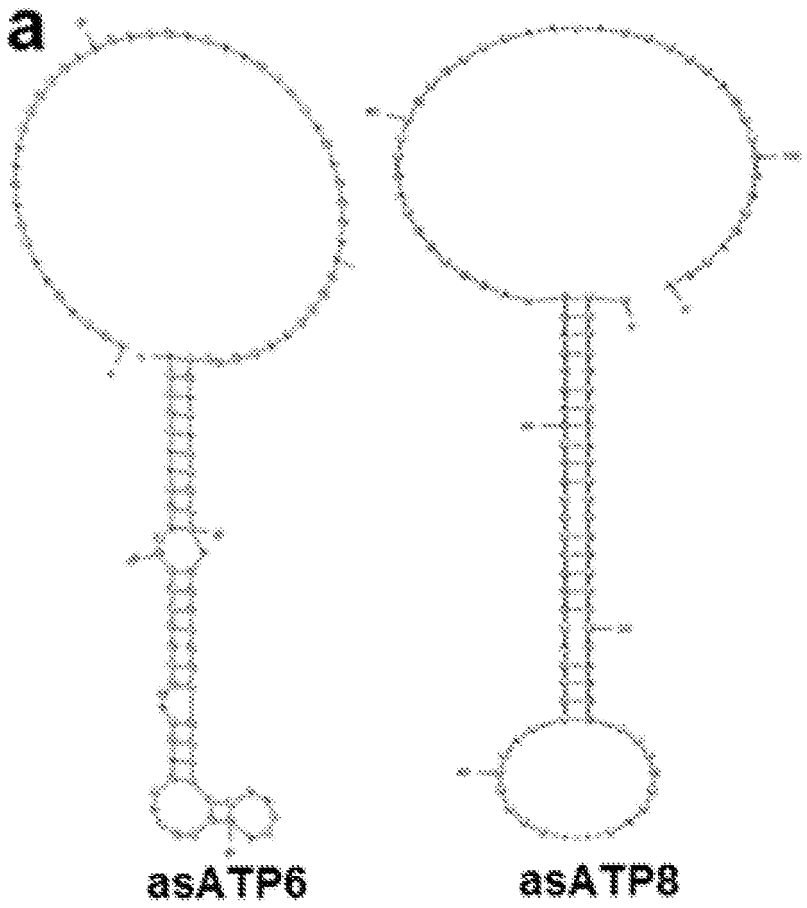


FIG. 4


a
asATP6
asATP8

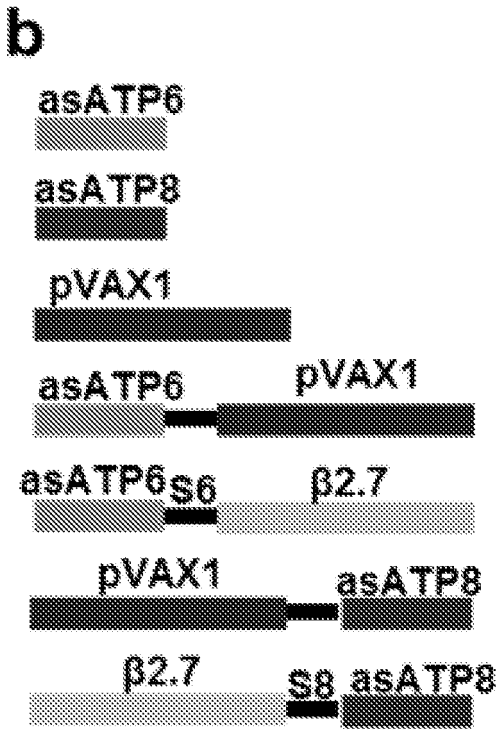

b
asATP6
asATP8
pVAX1
asATP6
pVAX1
asATP6
S6
β2.7
pVAX1
asATP8
β2.7
S8
asATP8

FIG. 4 continued

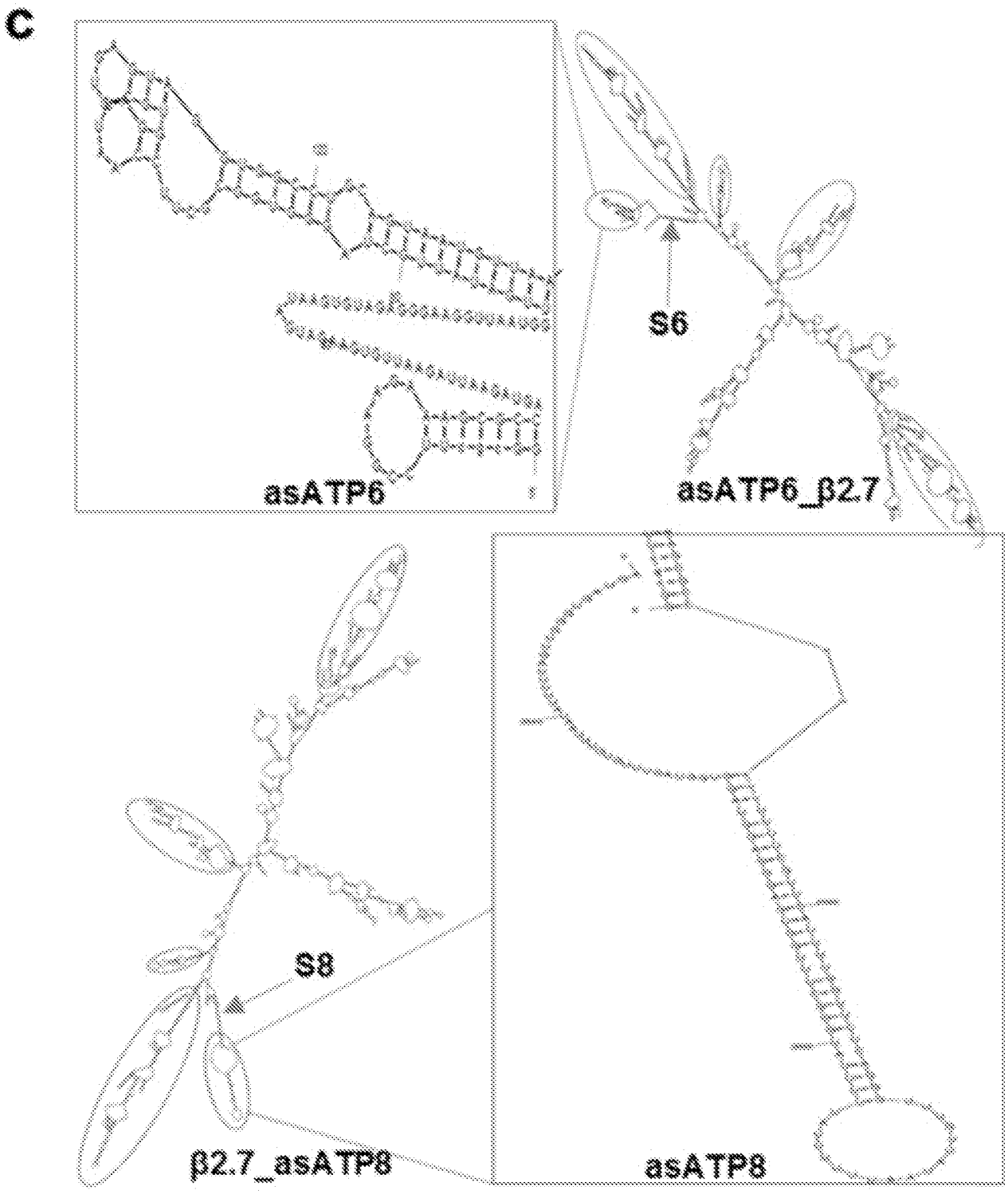


FIG. 4 continued

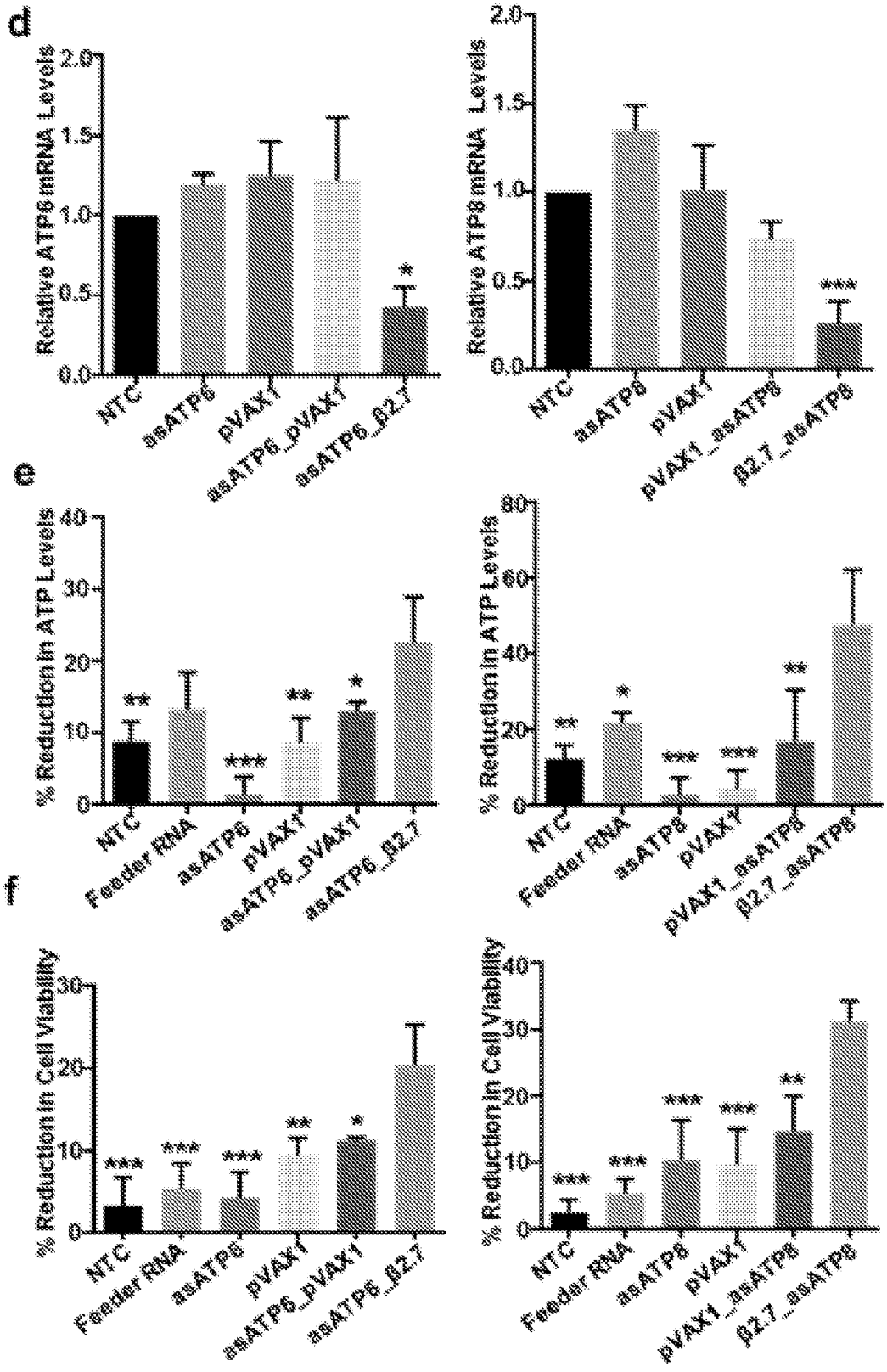


FIG. 5

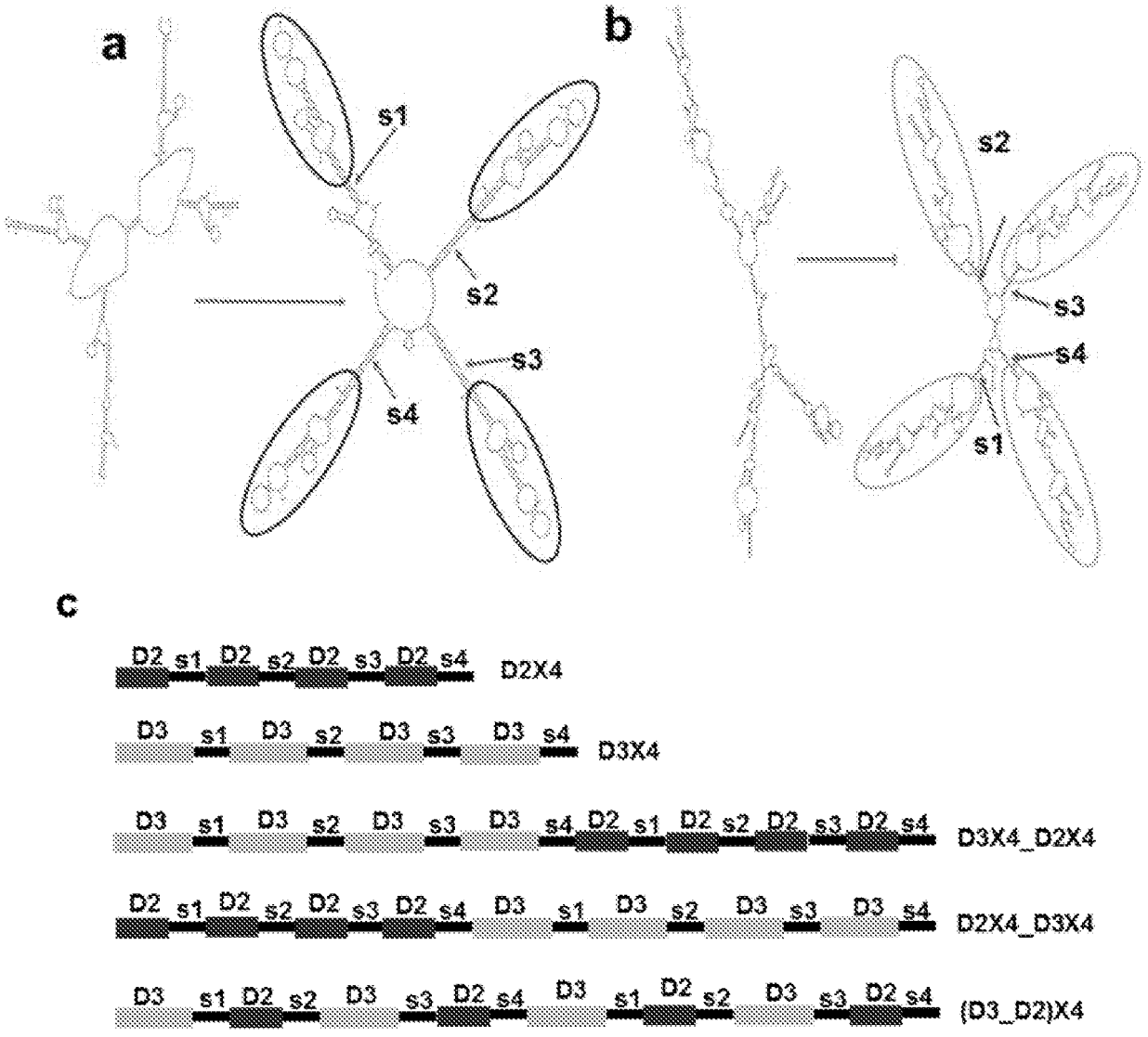

a
s1
s2
s3
s4
b
s2
s3
s4
s1
c
D2 s1 D2 s2 D2 s3 D2 s4 D2X4
D3 s1 D3 s2 D3 s3 D3 s4 D3X4
D3 s1 D3 s2 D3 s3 D3 s4 D2 s1 D2 s2 D2 s3 D2 s4 D3X4_D2X4
D2 s1 D2 s2 D2 s3 D2 s4 D3 s1 D3 s2 D3 s3 D3 s4 D2X4_D3X4
D3 s1 D2 s2 D3 s3 D2 s4 D3 s1 D2 s2 D3 s3 D2 s4 (D3_D2)X4
d
Median CT
No. of molecules
D2X4
β2.7
D3X4
D3X4_D2X4
D2X4_D3X4
(D3_D2)X4

FIG. 5 continued

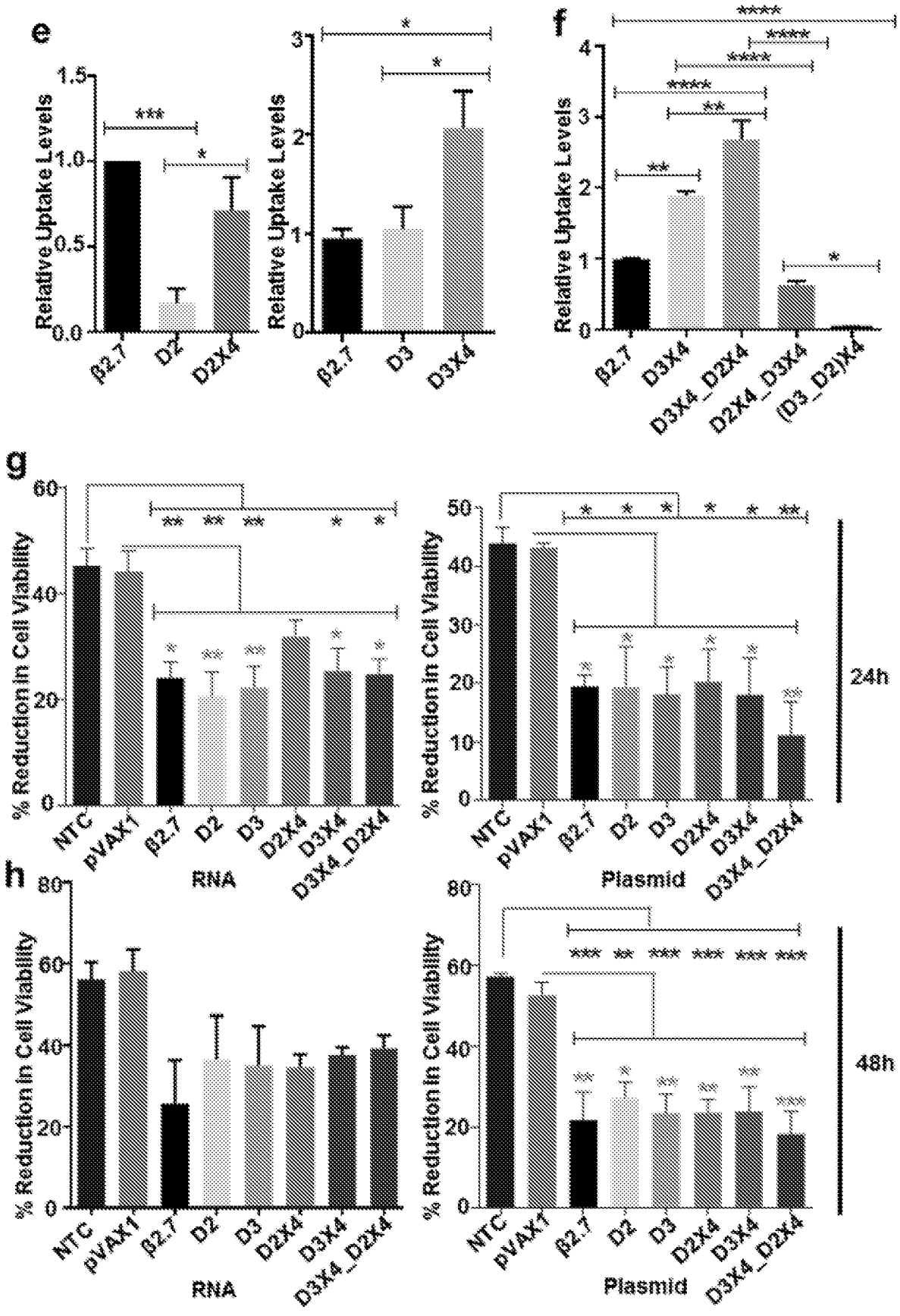


FIG. 6

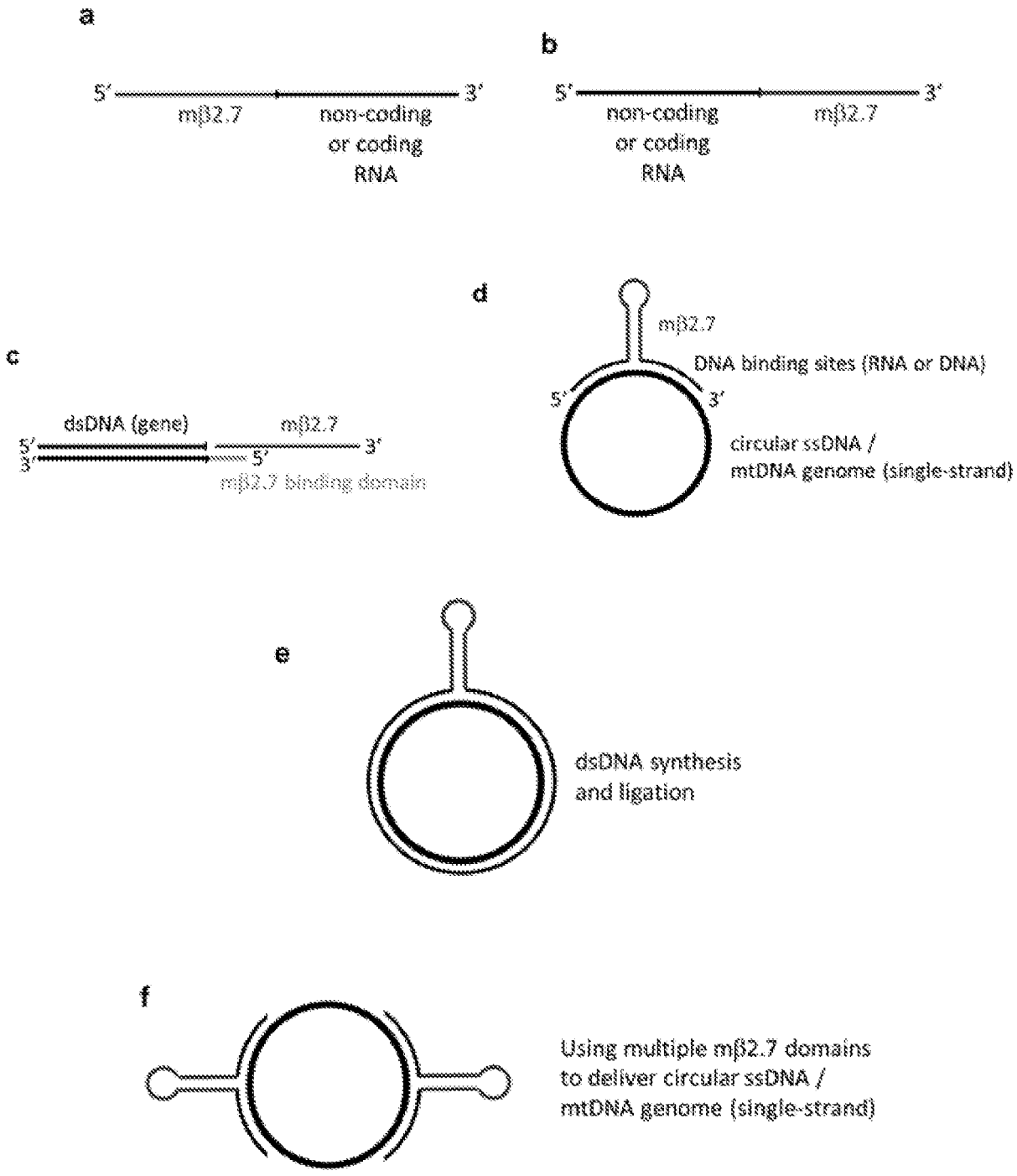

a
5'
3'
mβ2.7
non-coding
or coding
RNA
b
5'
3'
non-coding
or coding
RNA
mβ2.7
c
dsDNA (gene)
mβ2.7
5'
3'
5'
3'
mβ2.7 binding domain
d
mβ2.7
DNA binding sites (RNA or DNA)
5'
3'
circular ssDNA /
mtDNA genome (single-strand)
e
dsDNA synthesis
and ligation
f
Using multiple mβ2.7 domains
to deliver circular ssDNA /
mtDNA genome (single-strand)

FIG. 7


CMV
T7
BspEI
Stop Codon
NheI
Toward 2β7 RNA
HindIII
SP6
SV40 Poly A
Spacer
KanaR
BclI
DraIII

FIG. 8

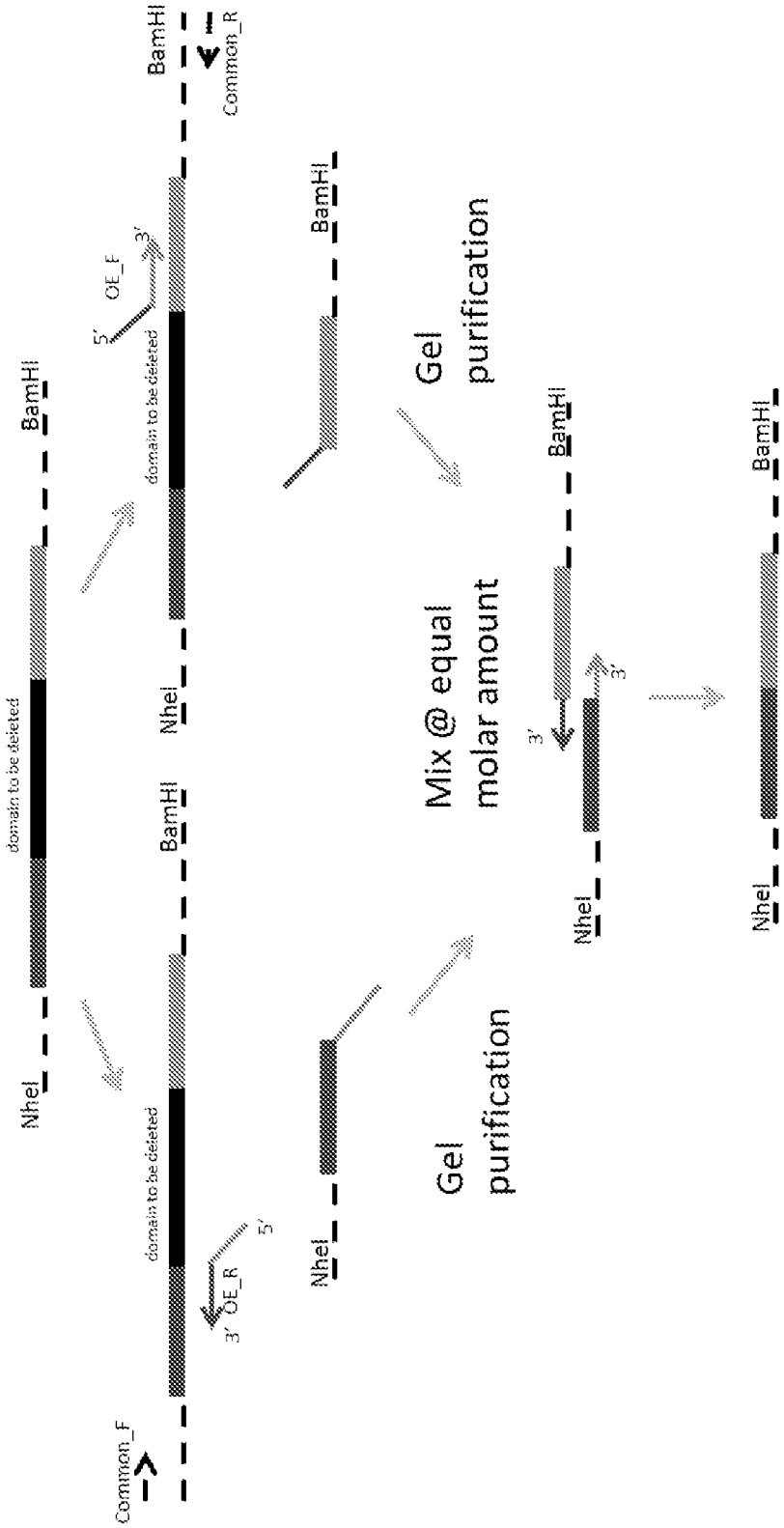

Common_F
Common_R
OE_F
OE_R
5'
3'
domain to be deleted
BamHI
NheI
Gel purification
Mix @ equal molar amount

FIG. 9

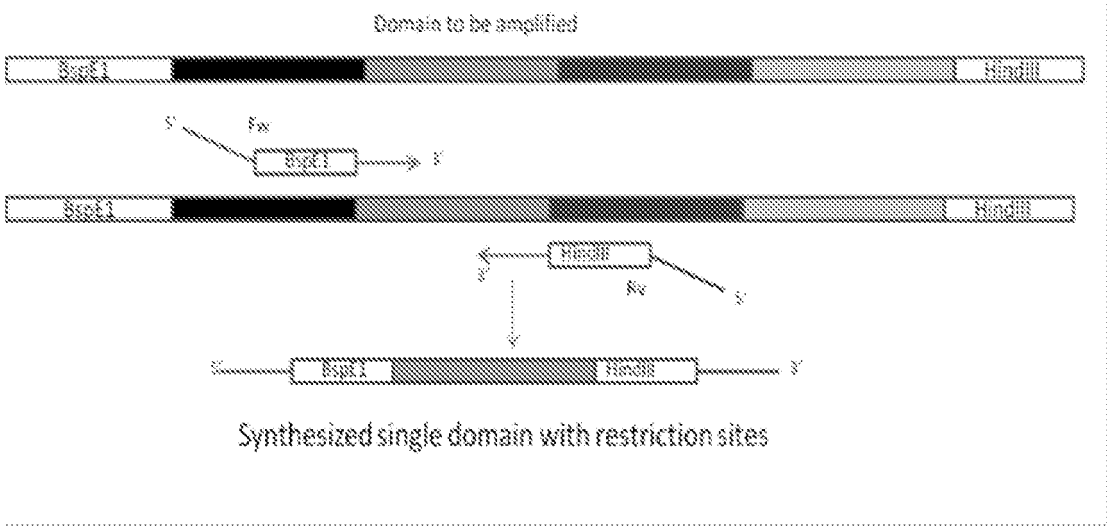


FIG. 10

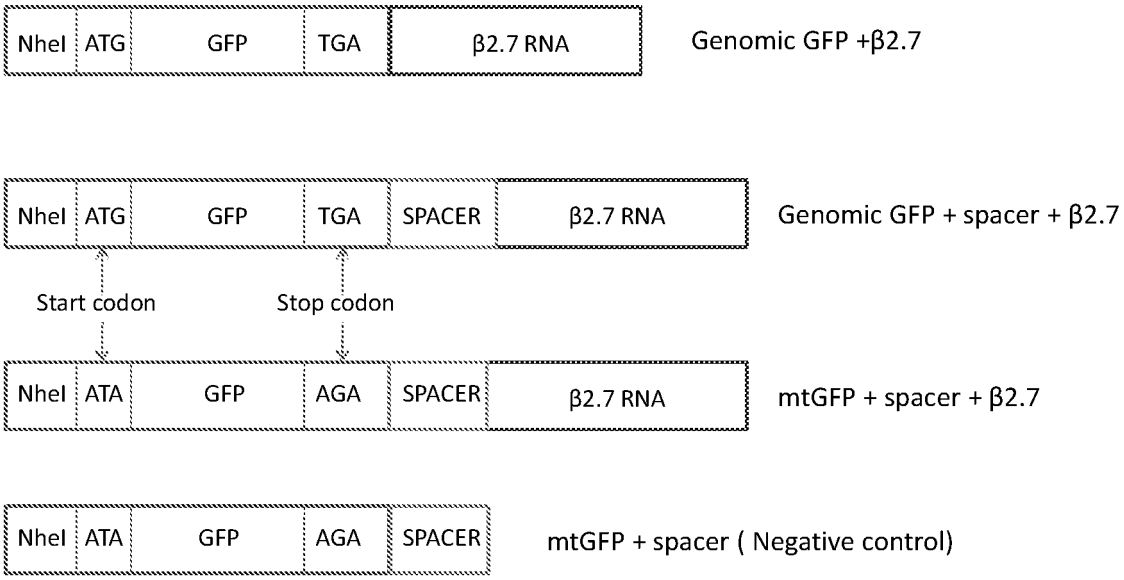


FIG. 11

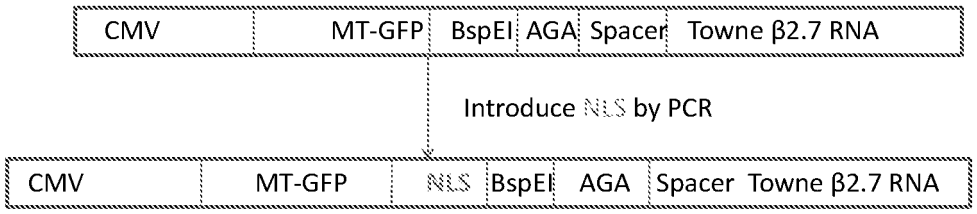


FIG. 12

Cloning Strategy

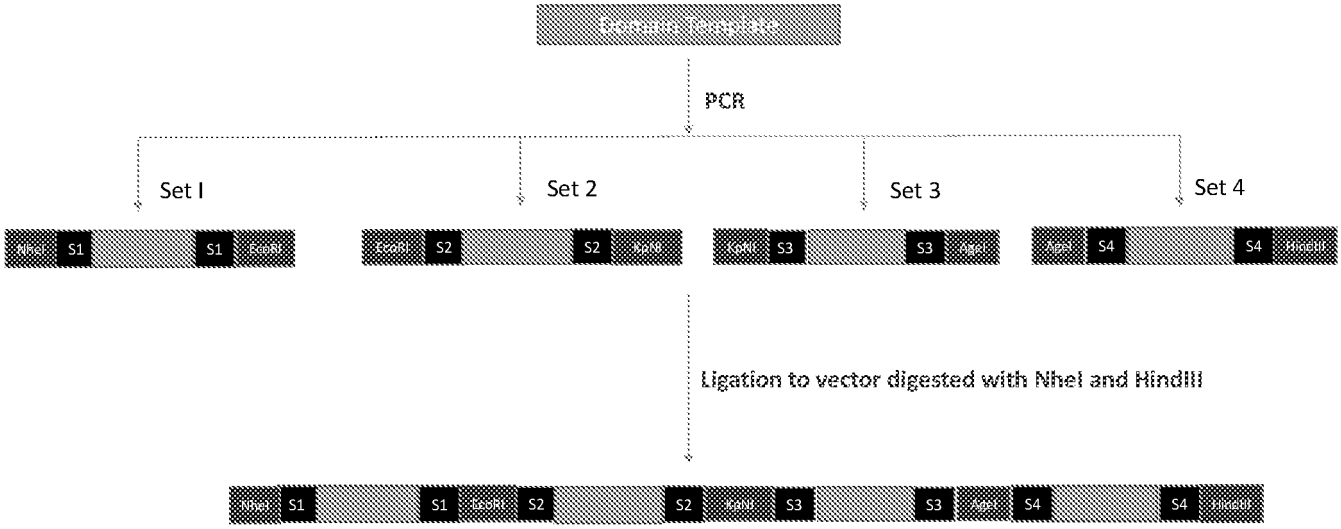


FIG. 13

D3X4_D2X4

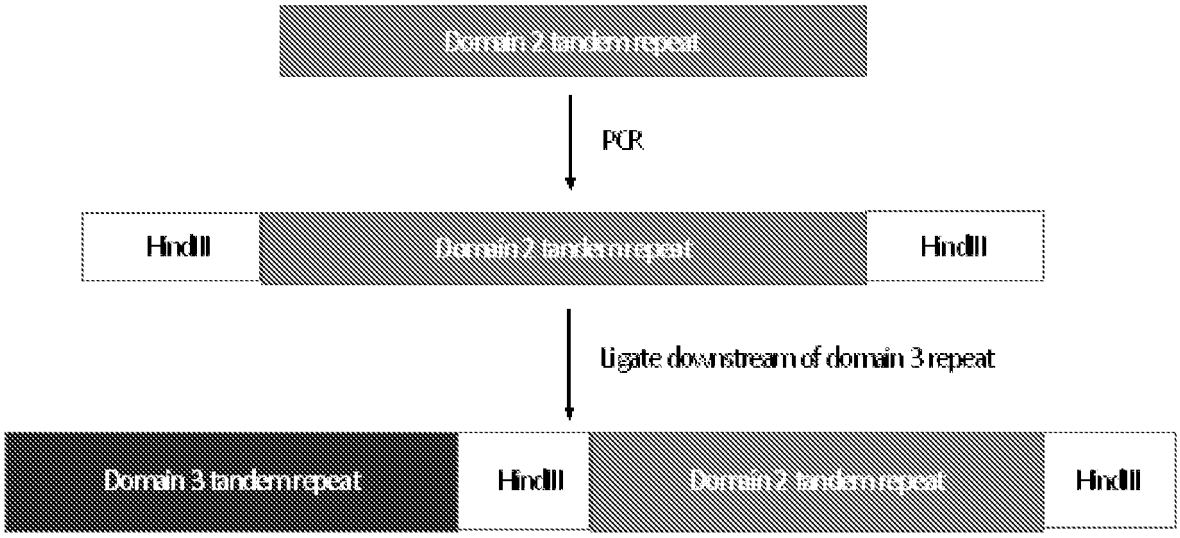


FIG. 13 continued

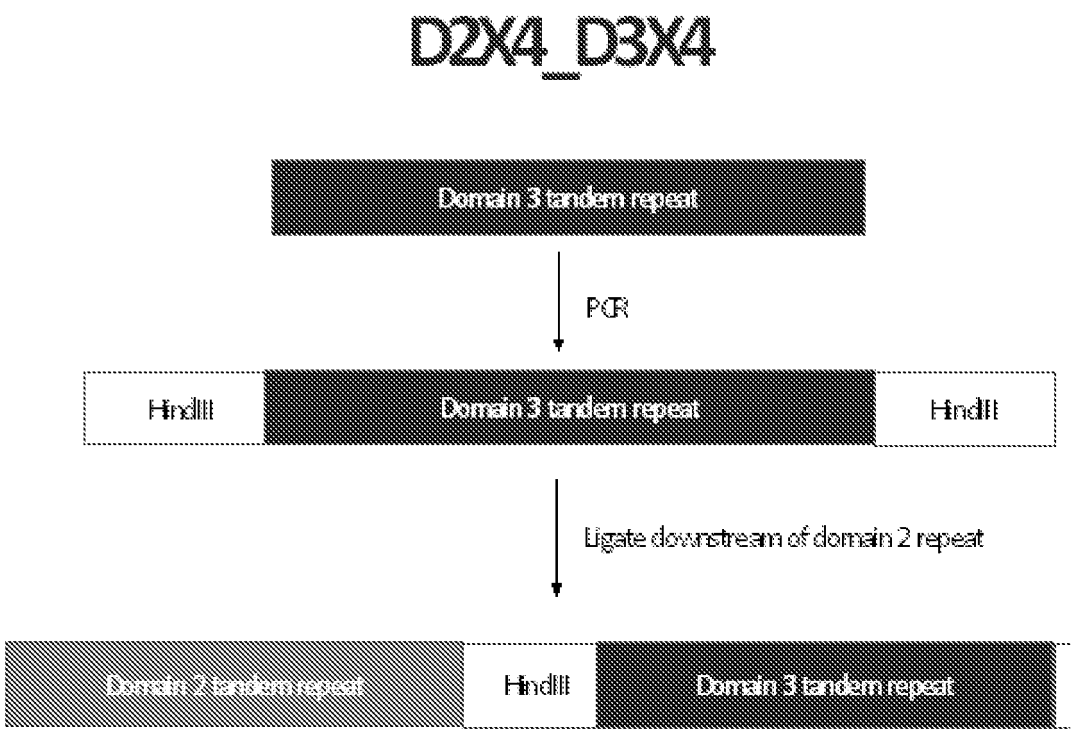


FIG. 14

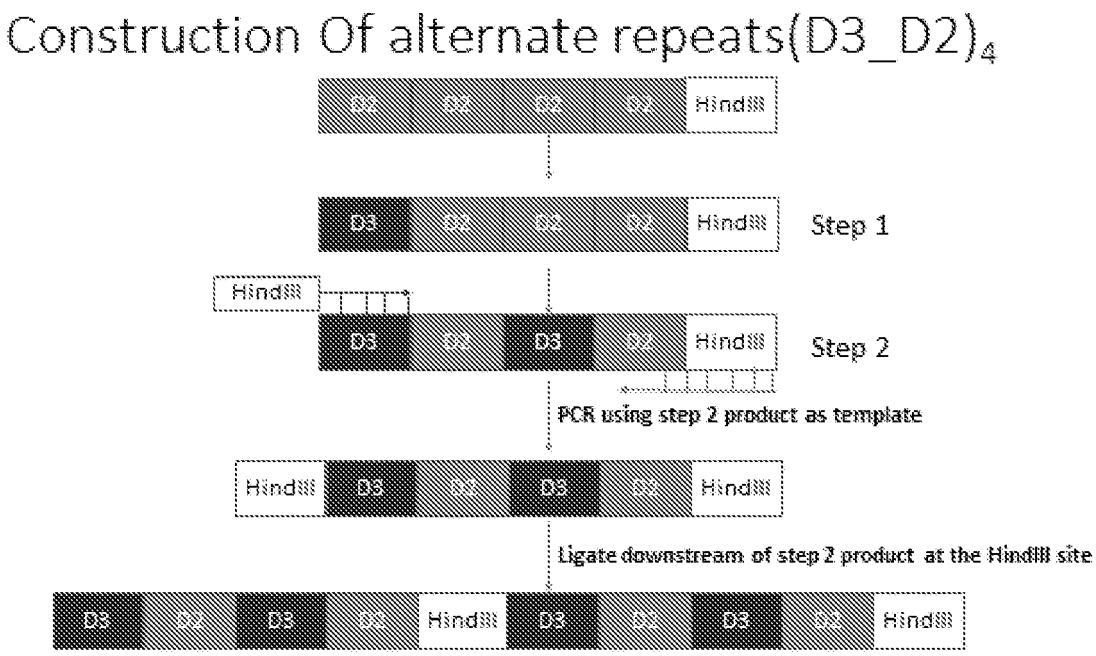


FIG. 15

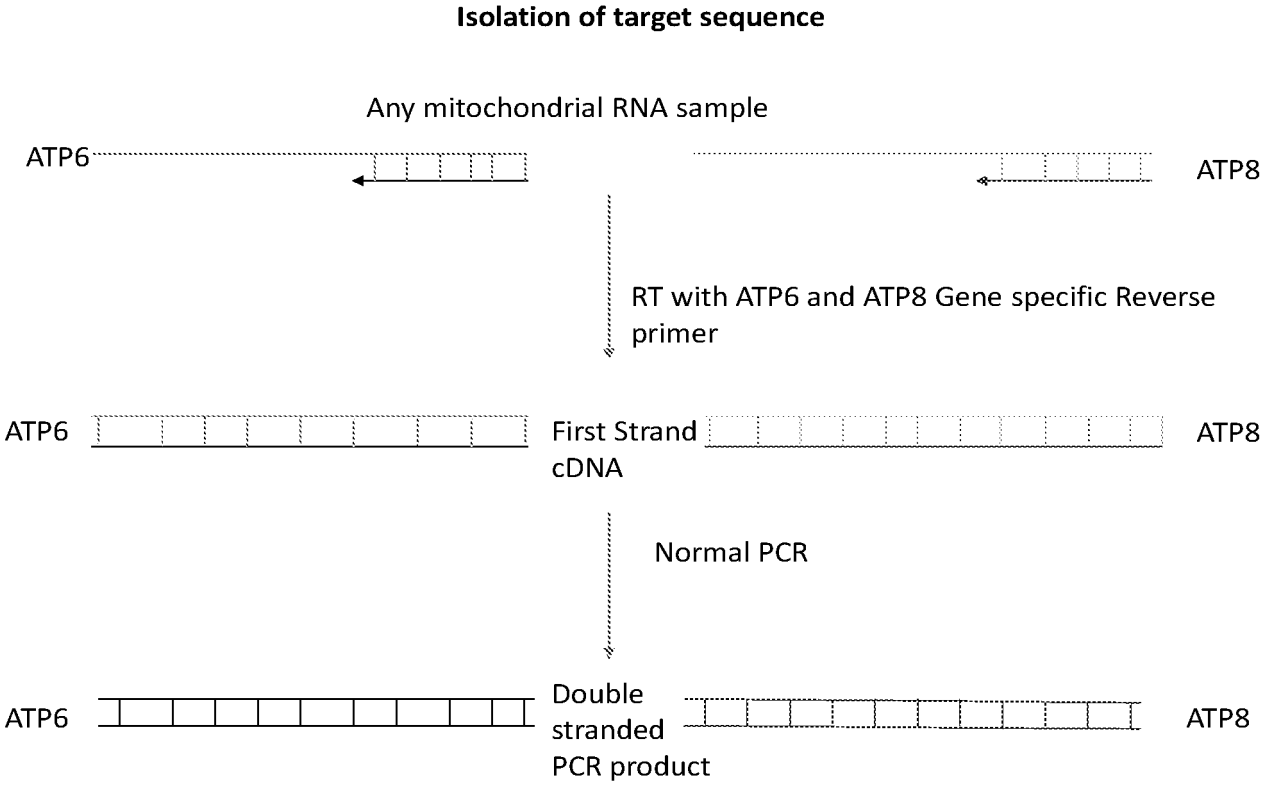

Isolation of target sequence
Any mitochondrial RNA sample
ATP6
ATP8
RT with ATP6 and ATP8 Gene specific Reverse primer
ATP6
First Strand cDNA
ATP8
Normal PCR
ATP6
Double stranded PCR product
ATP8

FIG. 16

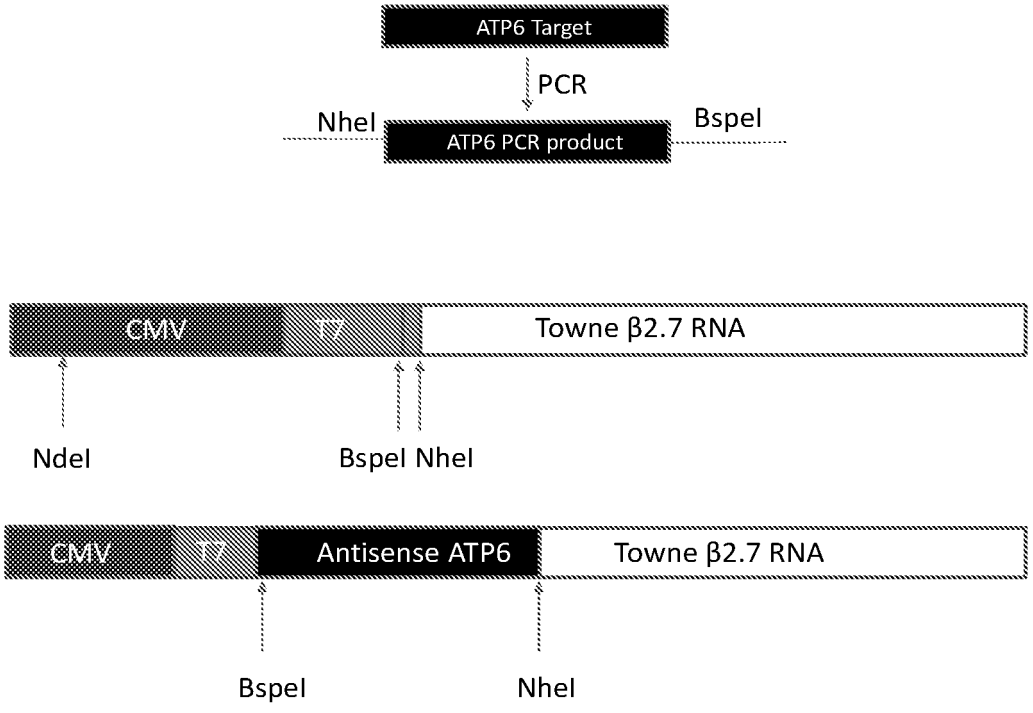

ATP6 Target
PCR
NheI
ATP6 PCR product
BspeI
CMV
T7
Towne β2.7 RNA
NdeI
BspeI NheI
CMV
T7
Antisense ATP6
Towne β2.7 RNA
BspeI
NheI

FIG. 17

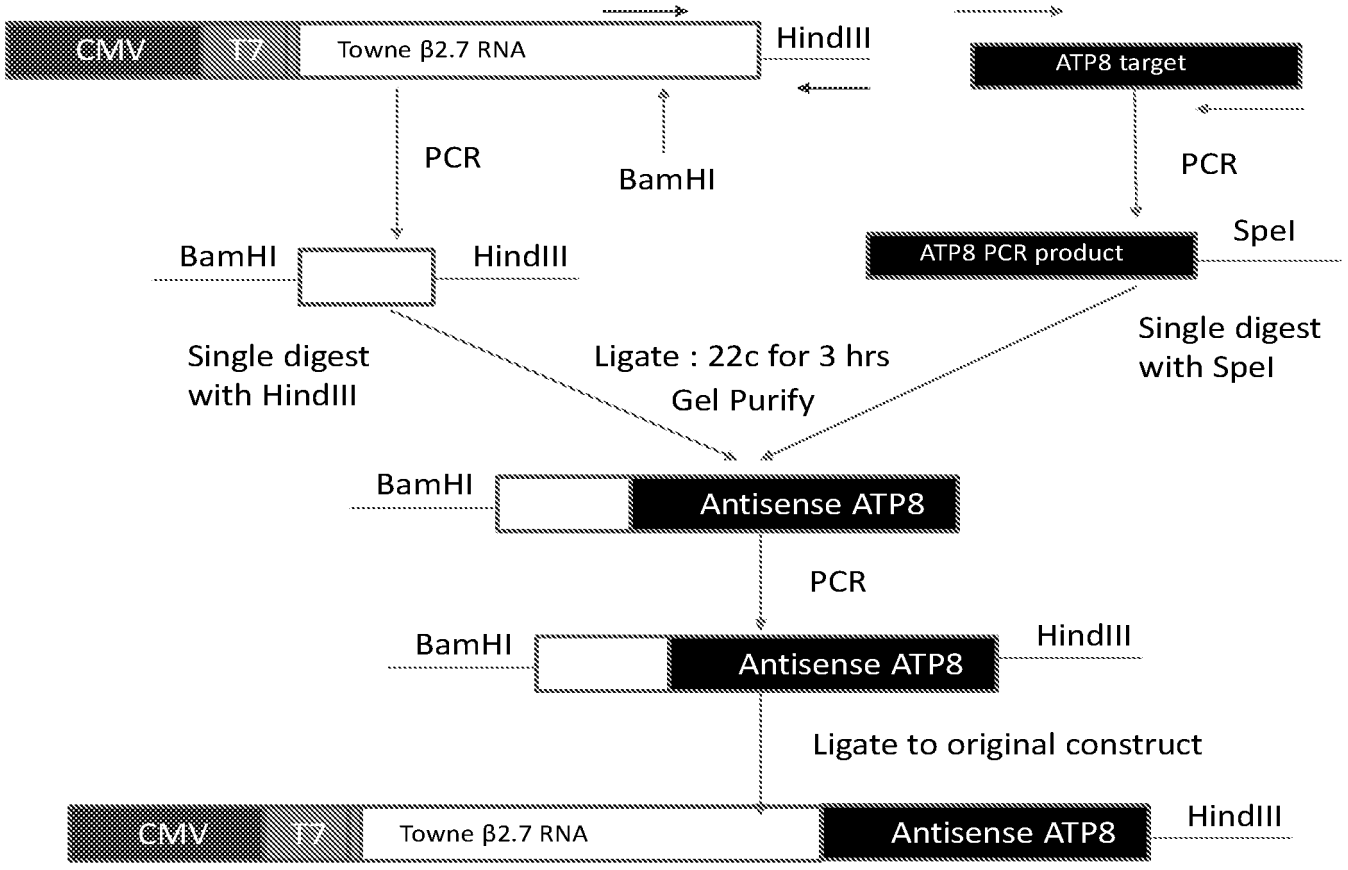

CMV
T7
Towne β2.7 RNA
HindIII
ATP8 target
PCR
BamHI
PCR
BamHI
HindIII
ATP8 PCR product
SpeI
Single digest with HindIII
Ligate : 22c for 3 hrs
Gel Purify
Single digest with SpeI
BamHI
Antisense ATP8
PCR
BamHI
Antisense ATP8
HindIII
Ligate to original construct
CMV
T7
Towne β2.7 RNA
Antisense ATP8
HindIII

FIG. 18

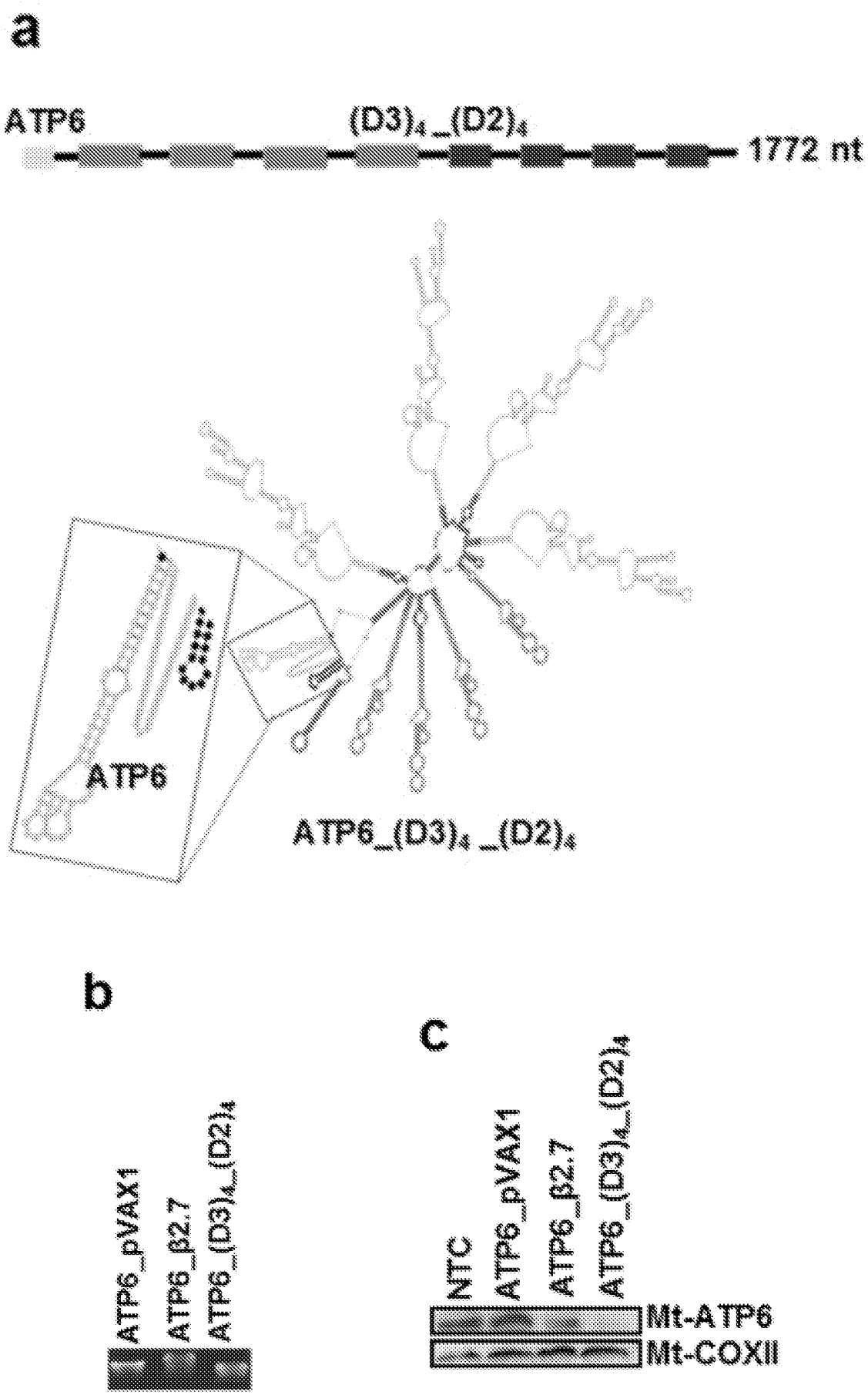

a
ATP6
(D3)4_(D2)4
1772 nt
ATP6
ATP6_(D3)4_(D2)4
b
ATP6_pVAX1
ATP6_β2.7
ATP6_(D3)4_(D2)4
c
NTC
ATP6_pVAX1
ATP6_β2.7
ATP6_(D3)4_(D2)4
Mt-ATP6
Mt-COXII

FIG. 18 continued

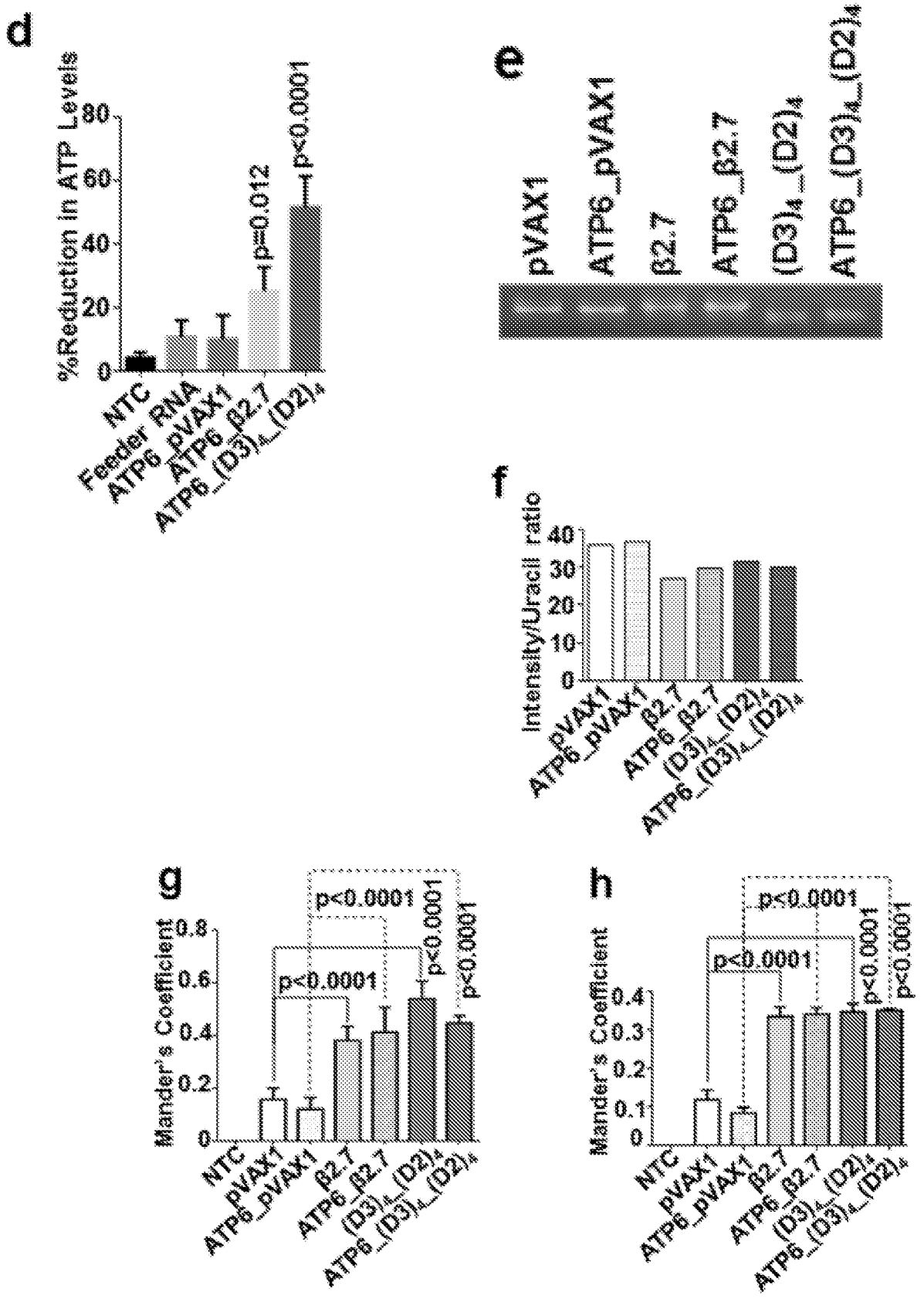


FIG. 18 continued

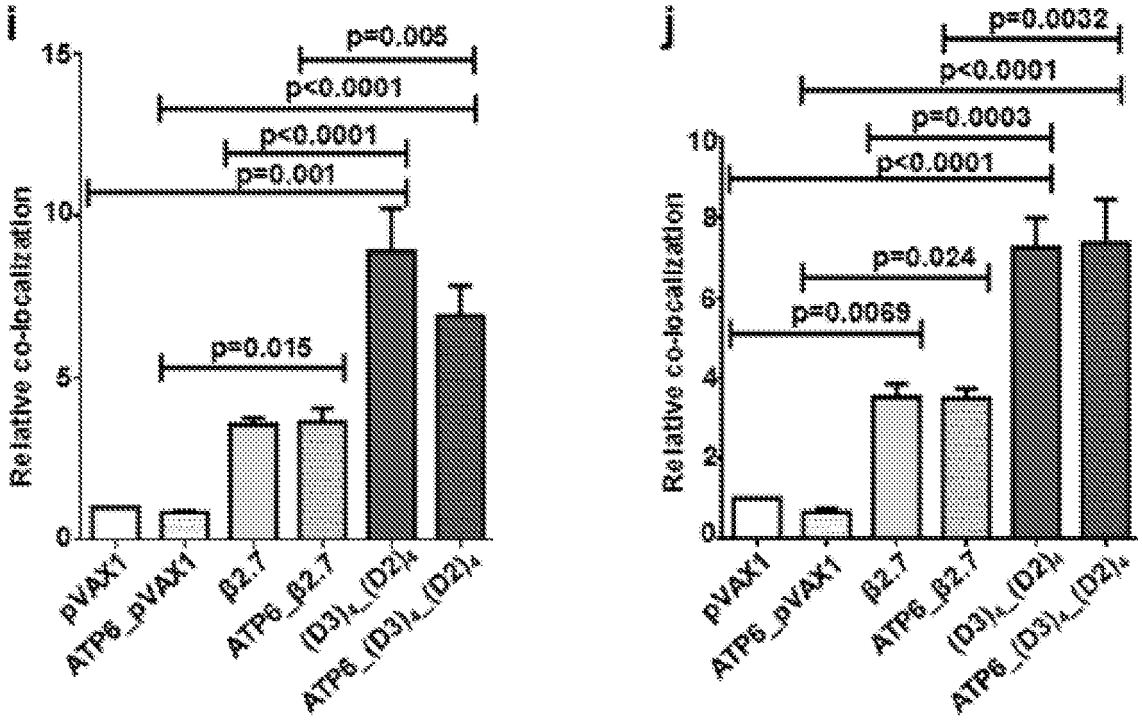


FIG. 19

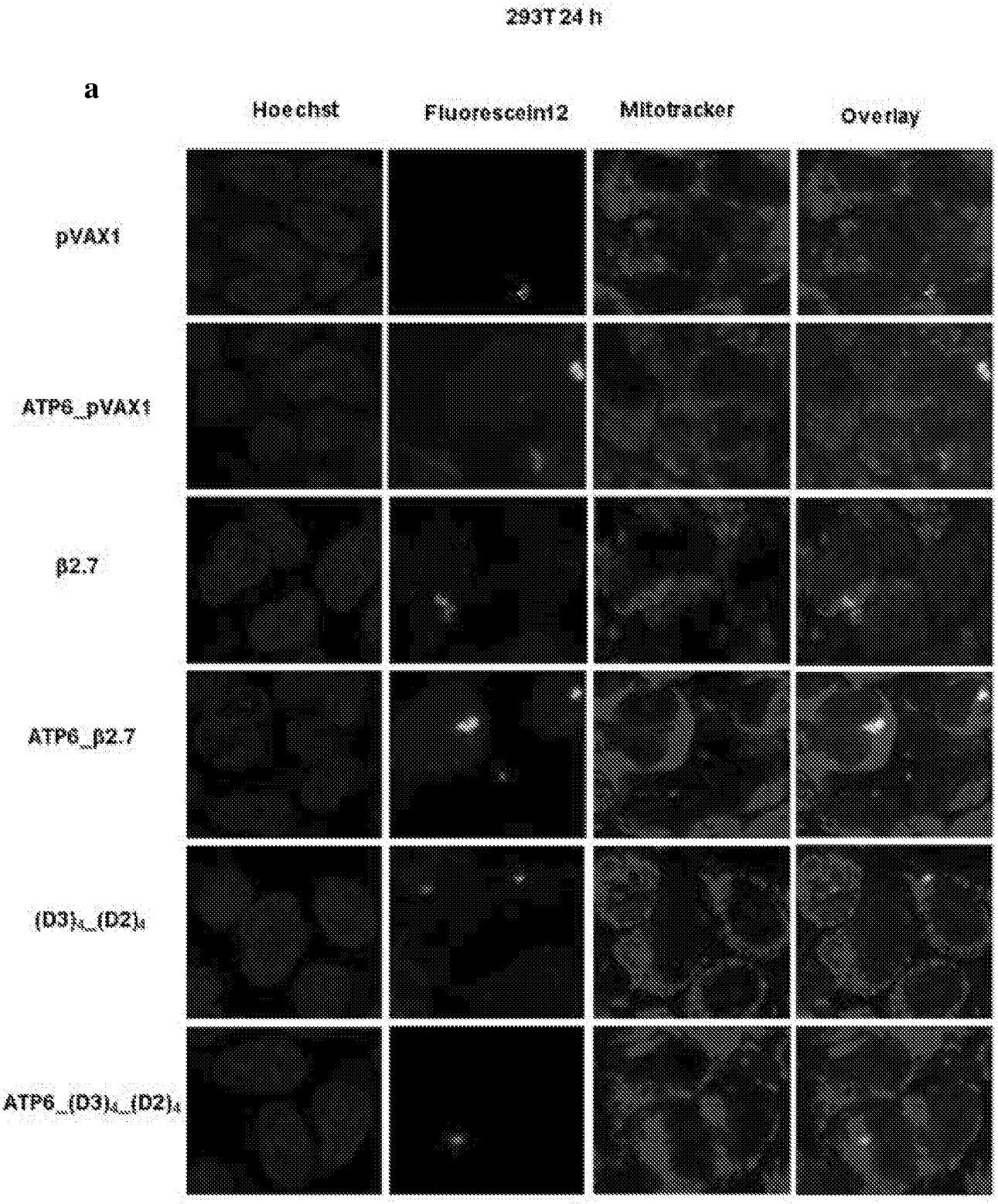

293T 24 h
a
Hoechst
Fluorescein12
Mitotracker
Overlay
pVAX1
ATP6_pVAX1
β2.7
ATP6_β2.7
(D3)k_(D2)k
ATP6_(D3)k_(D2)k

FIG. 19 continued

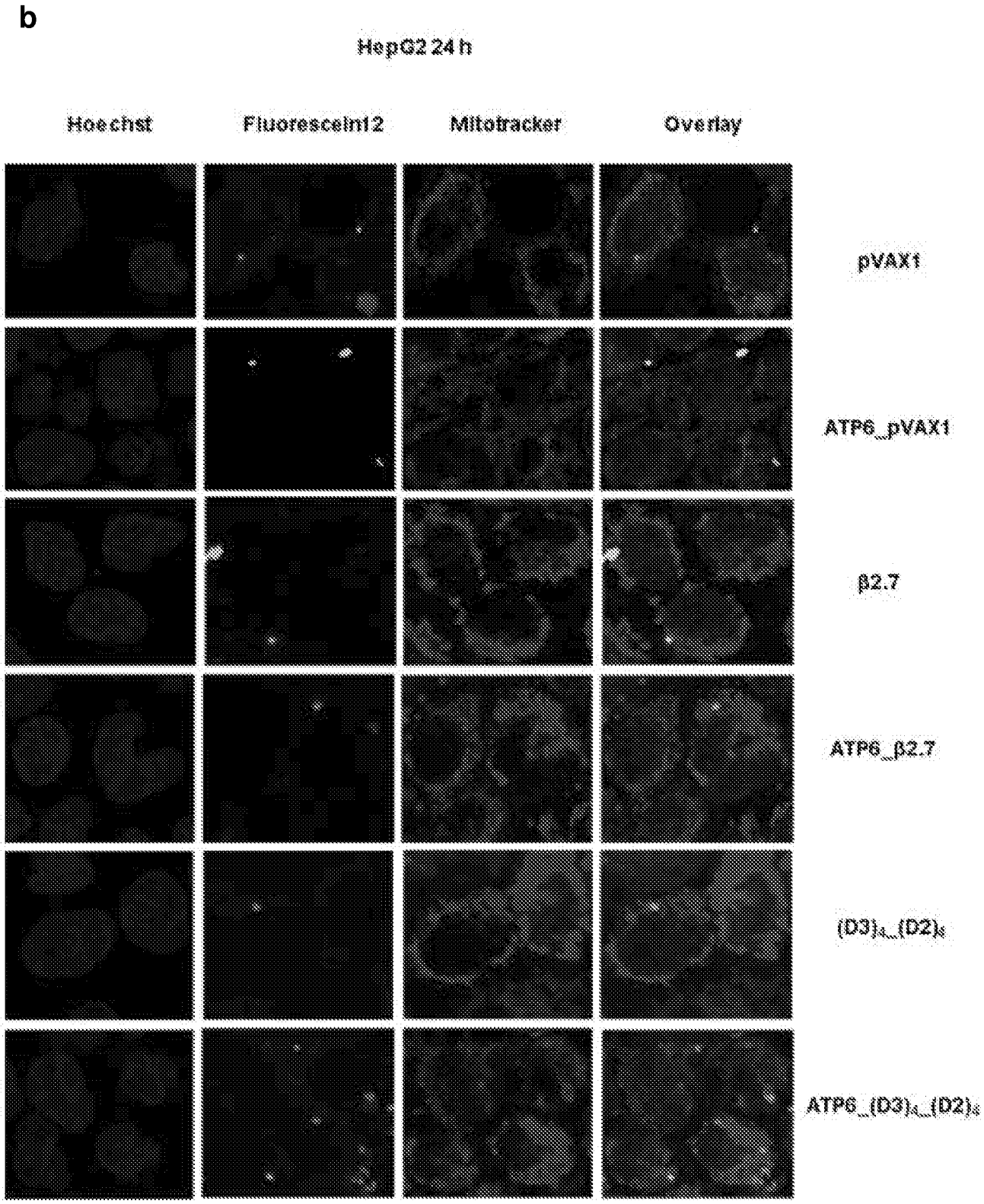


FIG. 20

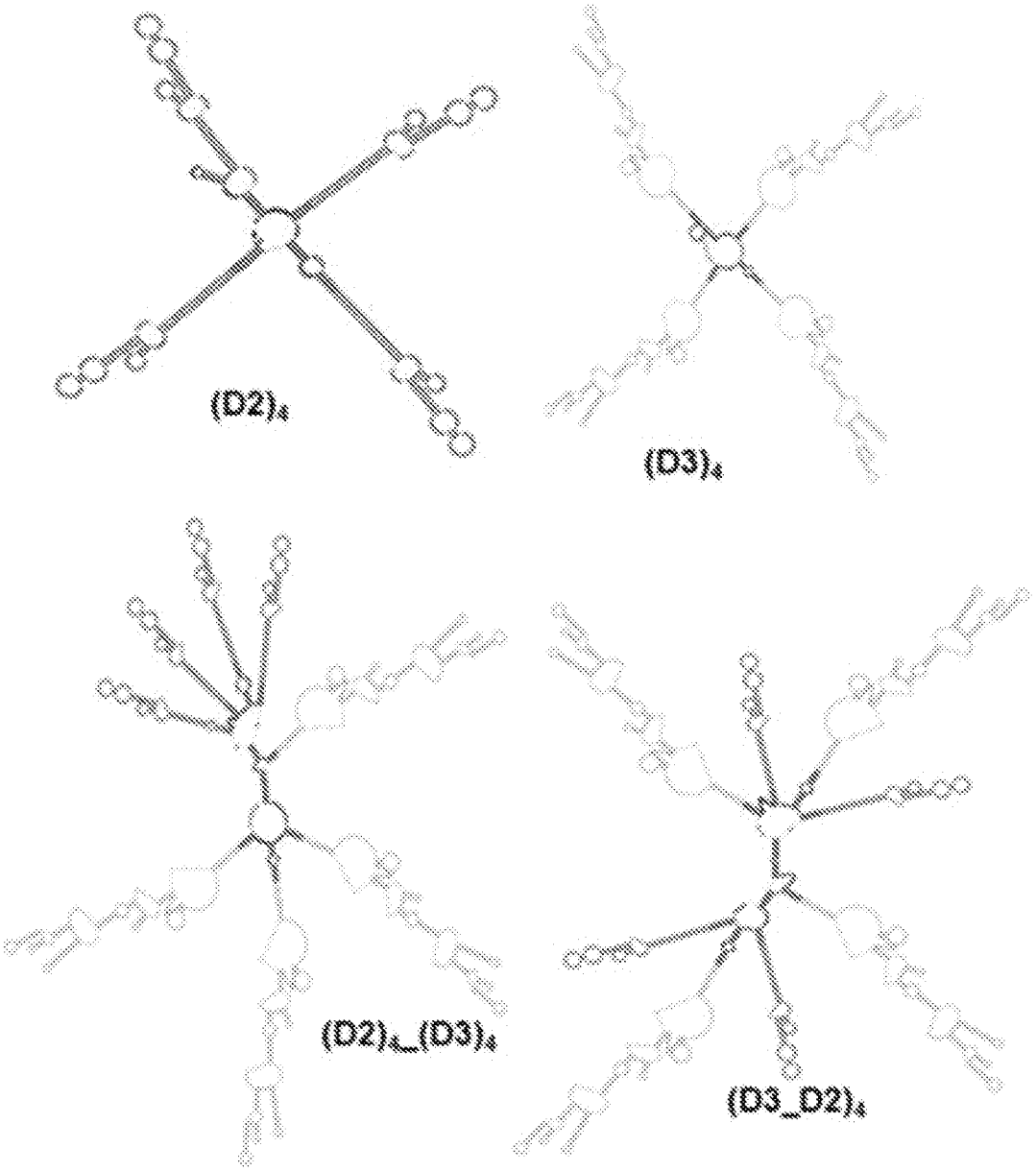


FIG. 20 continued

