FIG. 1

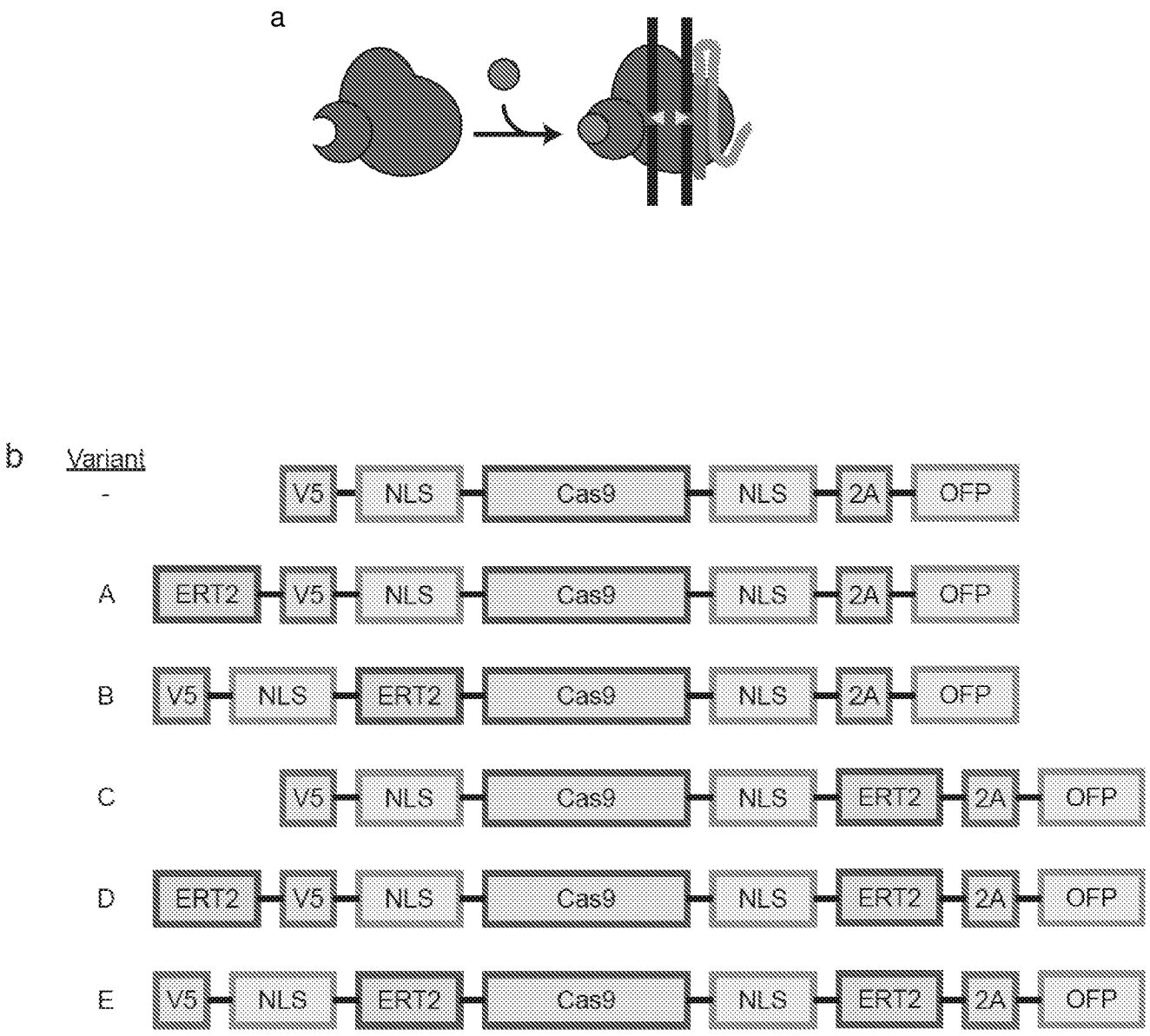


FIG. 1 continued

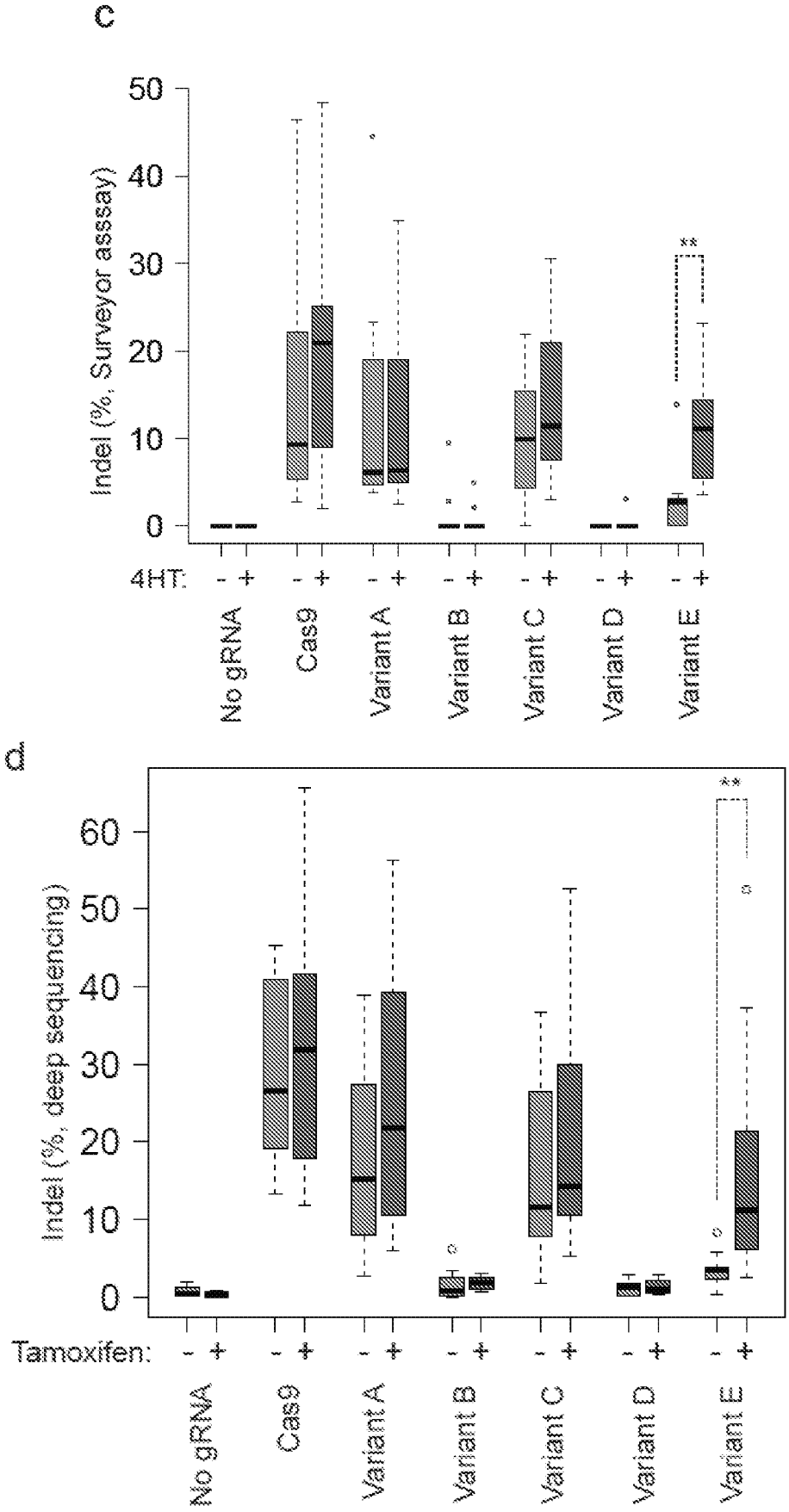


FIG. 2

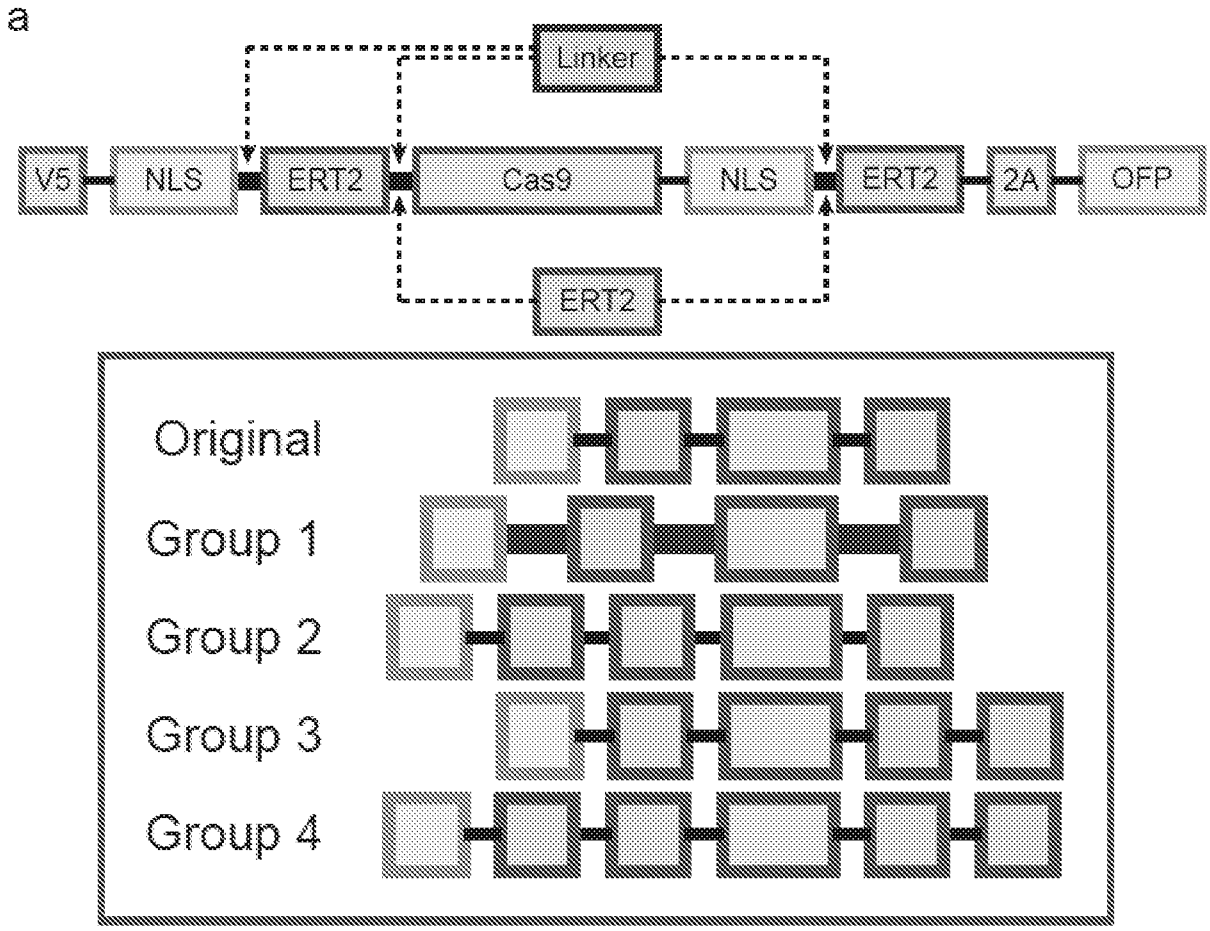

a
Linker
V5
NLS
ERT2
Cas9
NLS
ERT2
2A
OFP
ERT2
Original
Group 1
Group 2
Group 3
Group 4

FIG. 2 continued

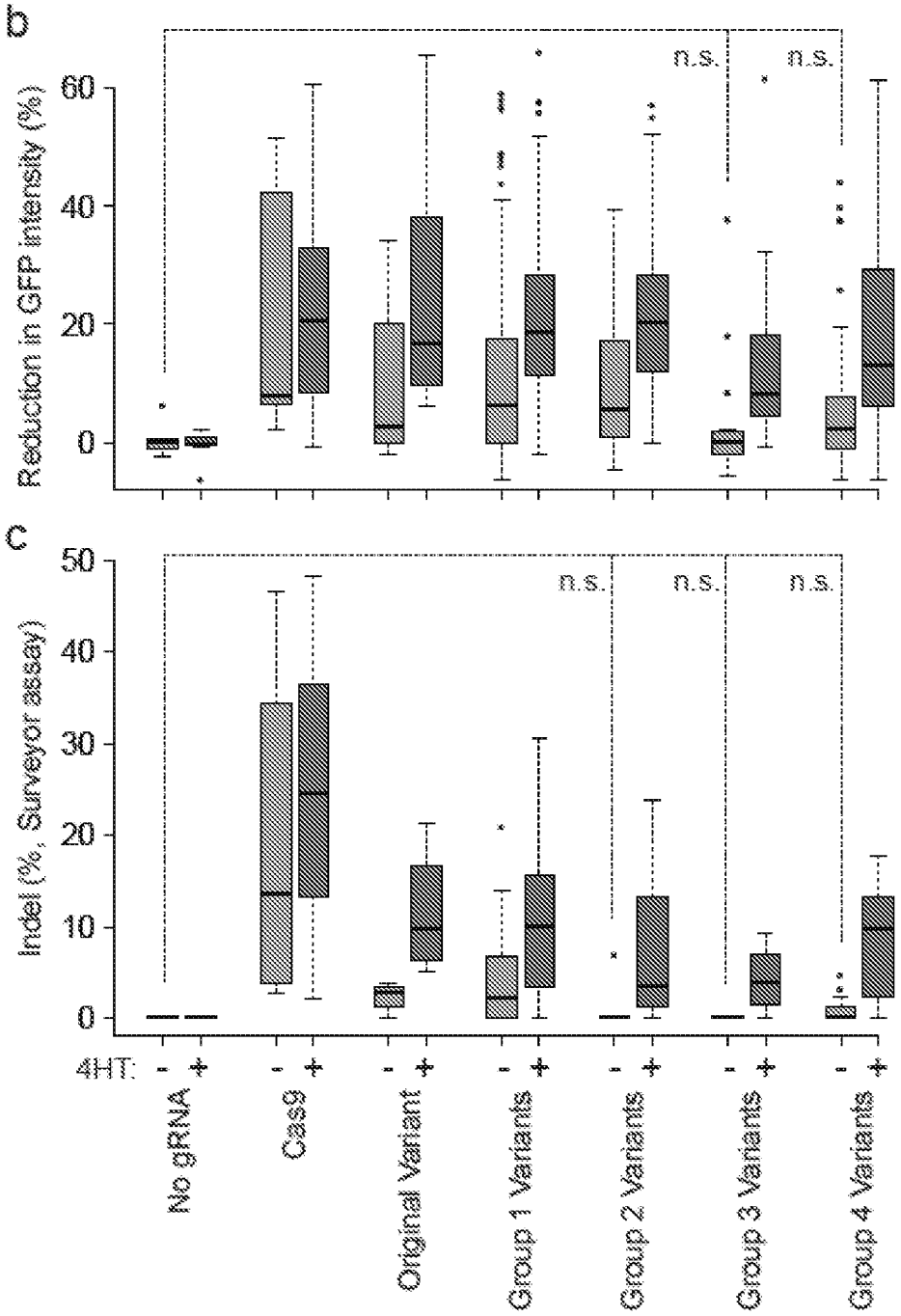


FIG. 2 continued

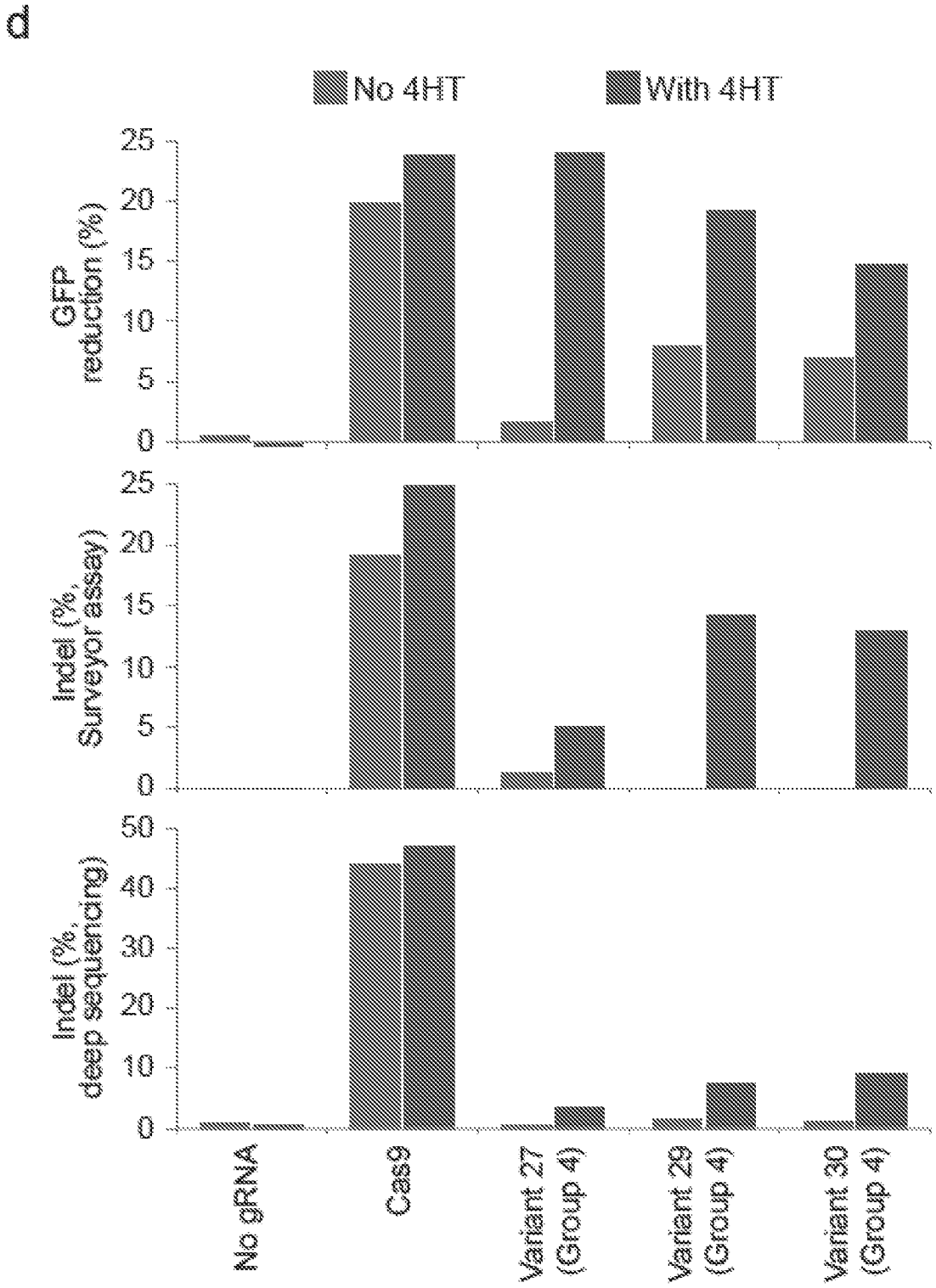


FIG. 3

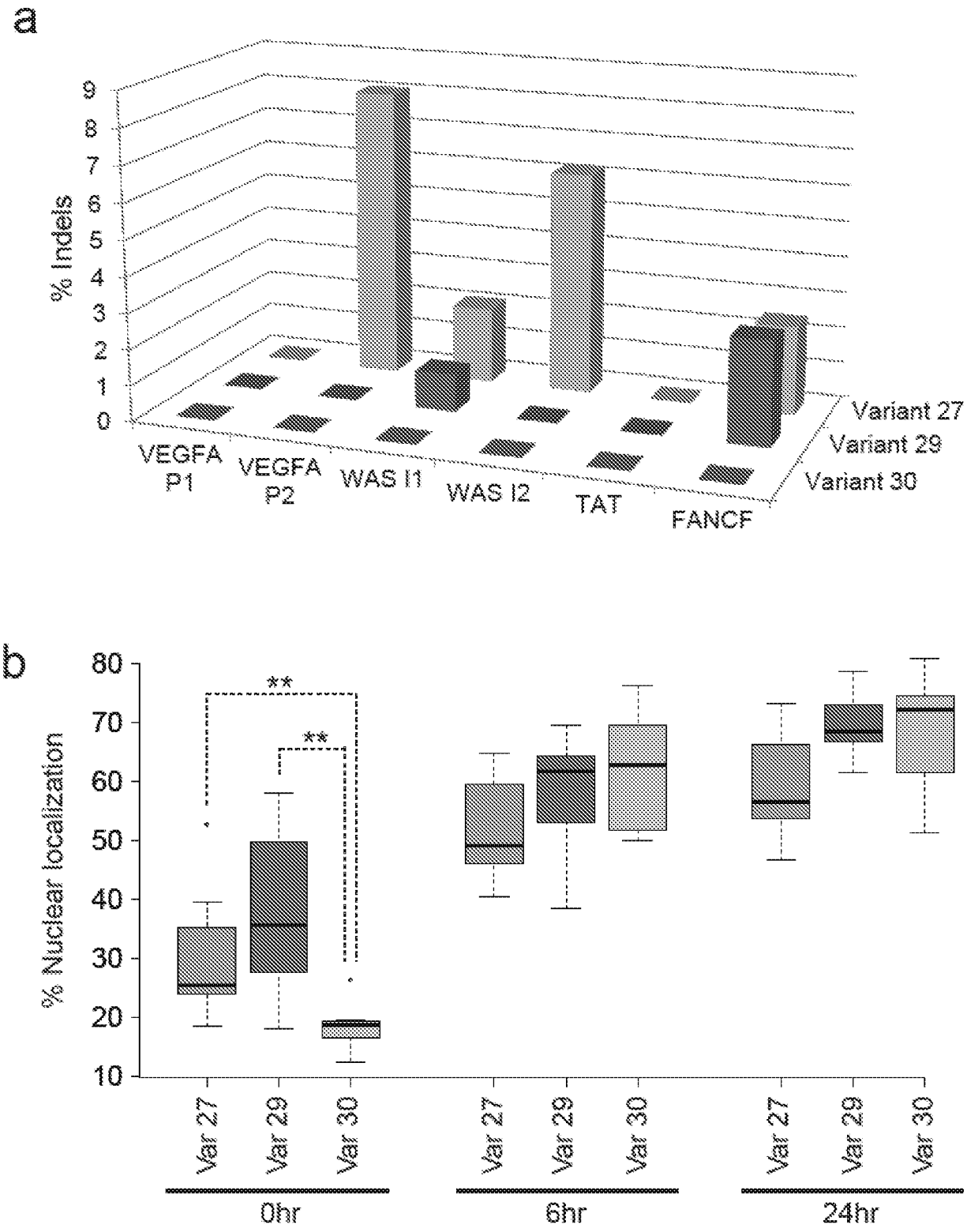

a
% Indels
0
1
2
3
4
5
6
7
8
9
VEGFA P1
VEGFA P2
WAS I1
WAS I2
TAT
FANCF
Variant 27
Variant 29
Variant 30
b
% Nuclear localization
10
20
30
40
50
60
70
80
**
**
Var 27
Var 29
Var 30
0hr
6hr
24hr

FIG. 3 continued

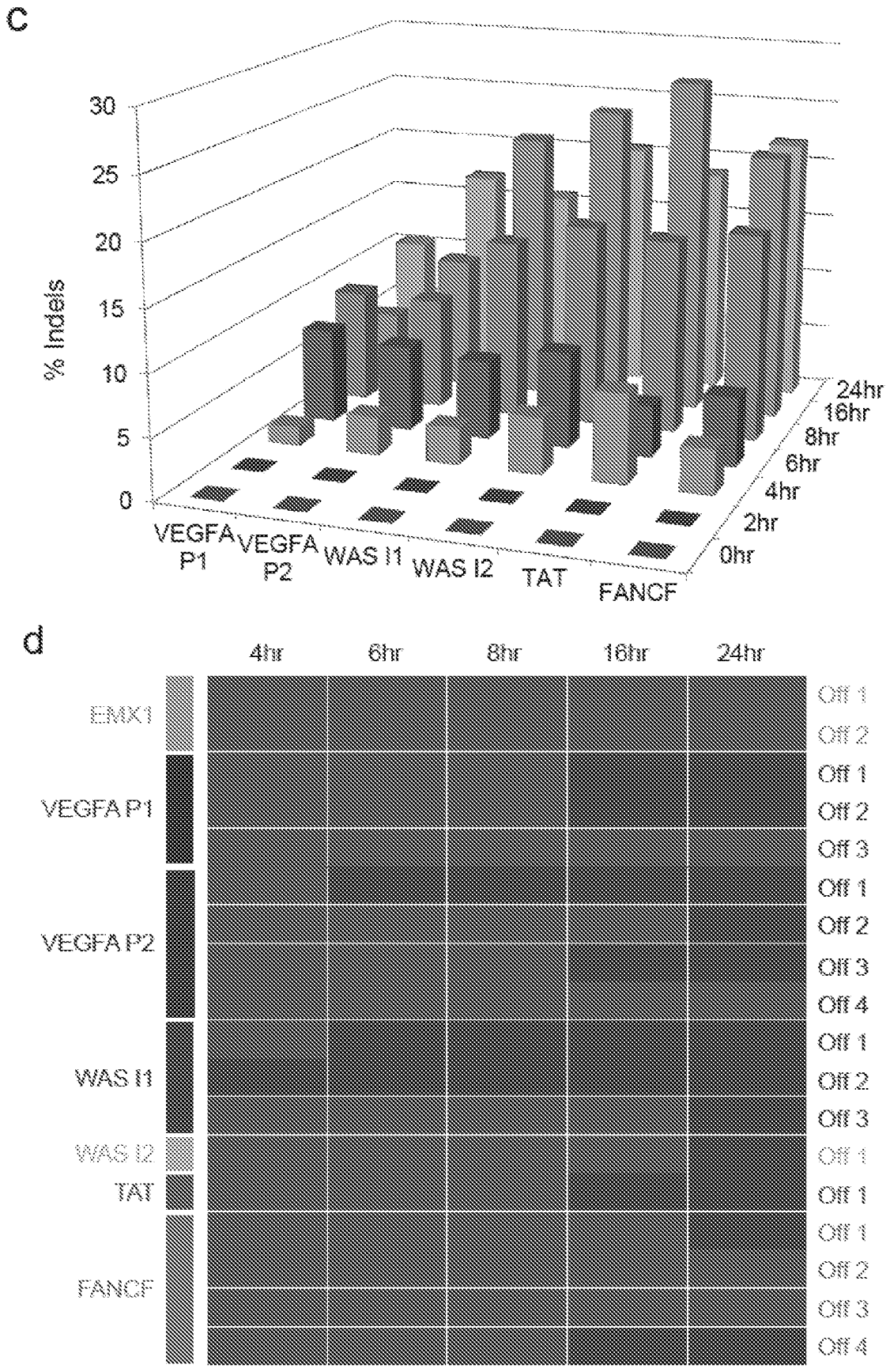


FIG. 4

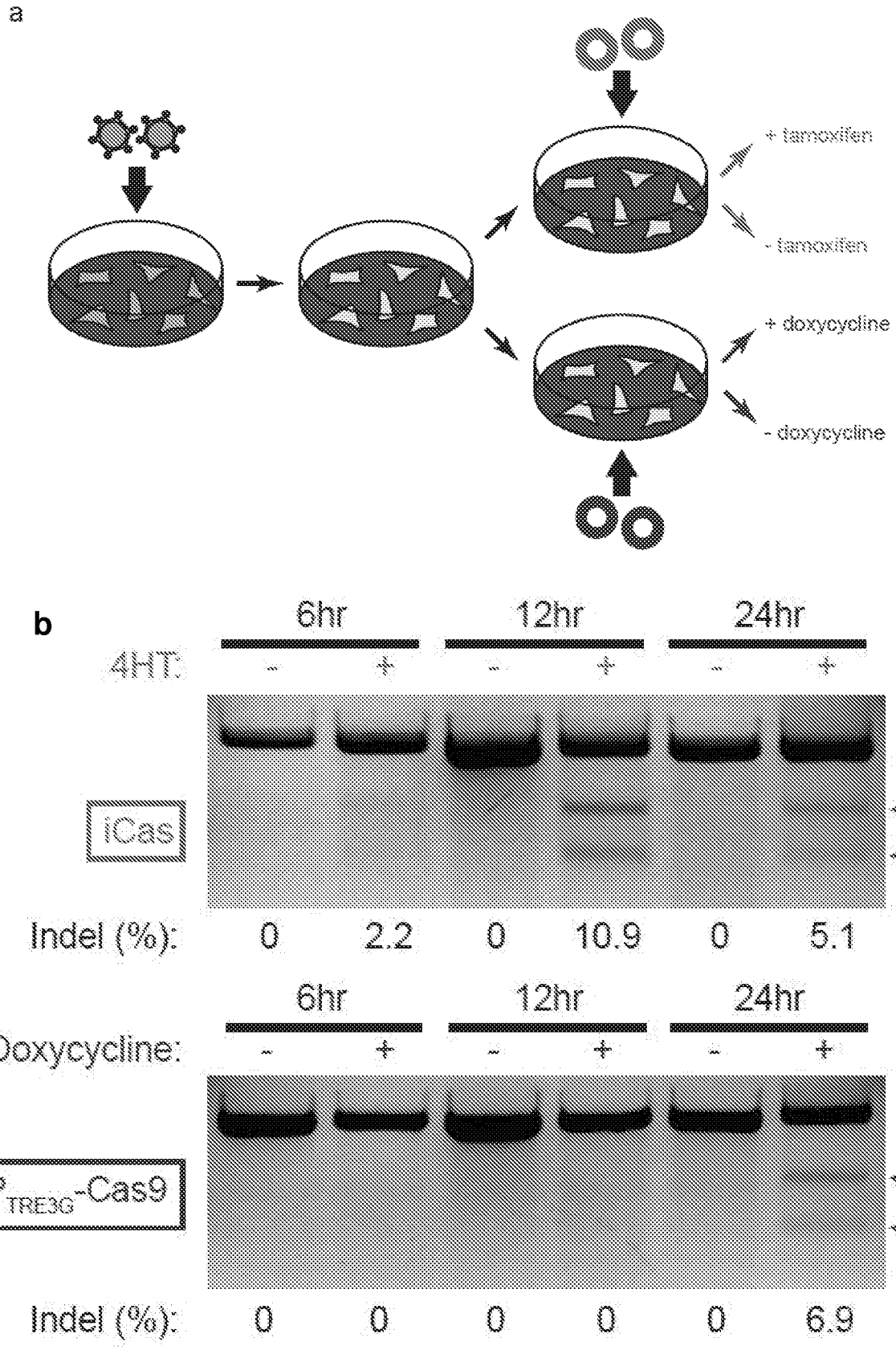
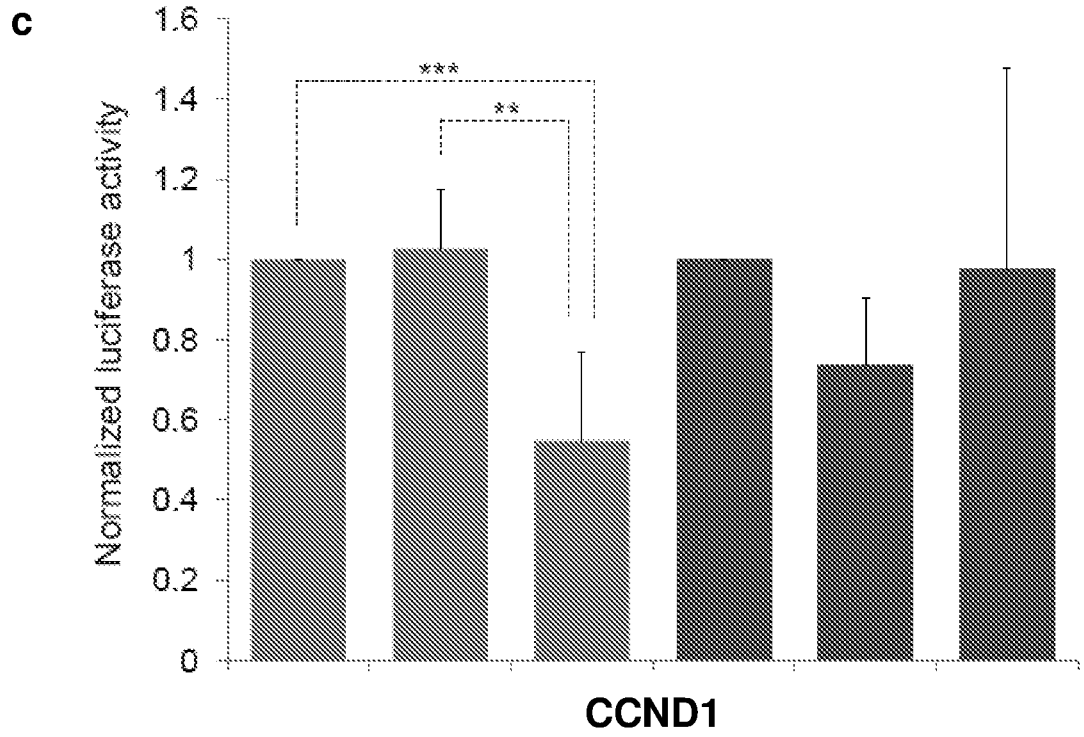

a
+ tamoxifen
- tamoxifen
+ doxycycline
- doxycycline
b
6hr
12hr
24hr
4HT:
- + - + - +
iCas
Indel (%): 0 2.2 0 10.9 0 5.1
6hr
12hr
24hr
Doxycycline:
- + - + - +
P TRE3G -Cas9
Indel (%): 0 0 0 0 0 6.9

FIG. 4 continued



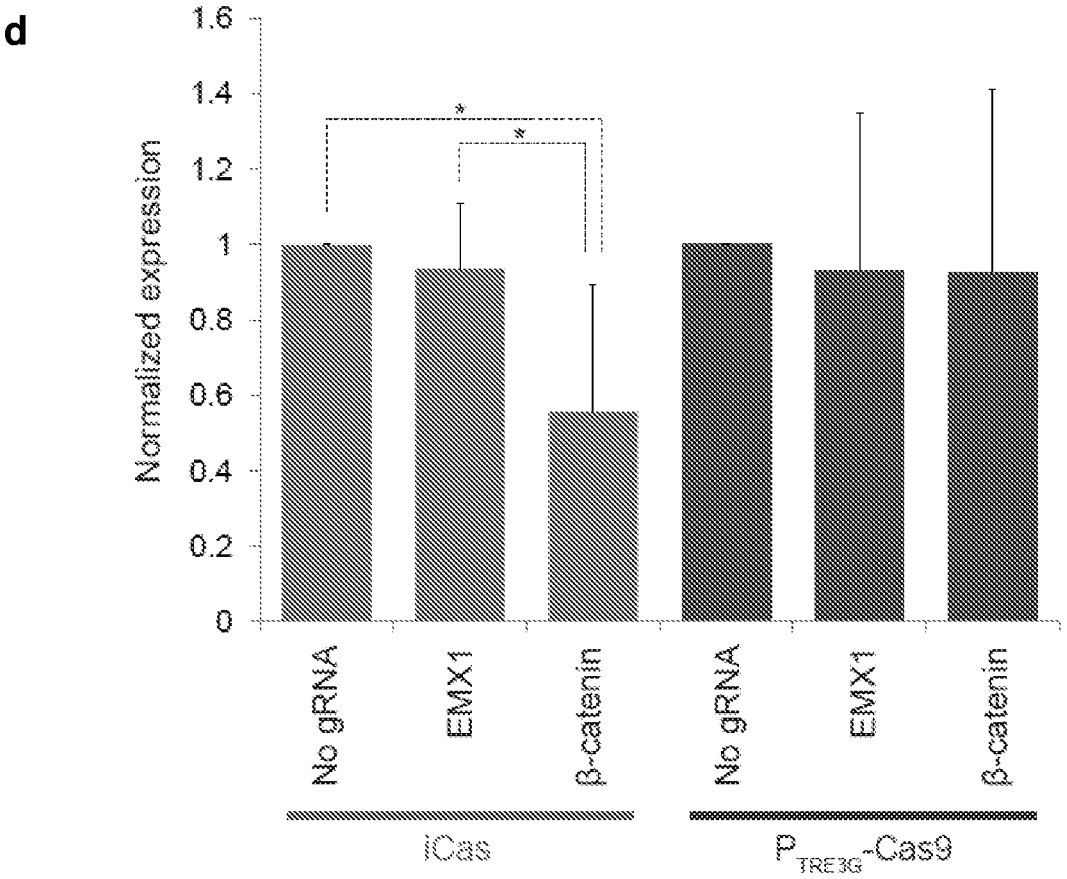
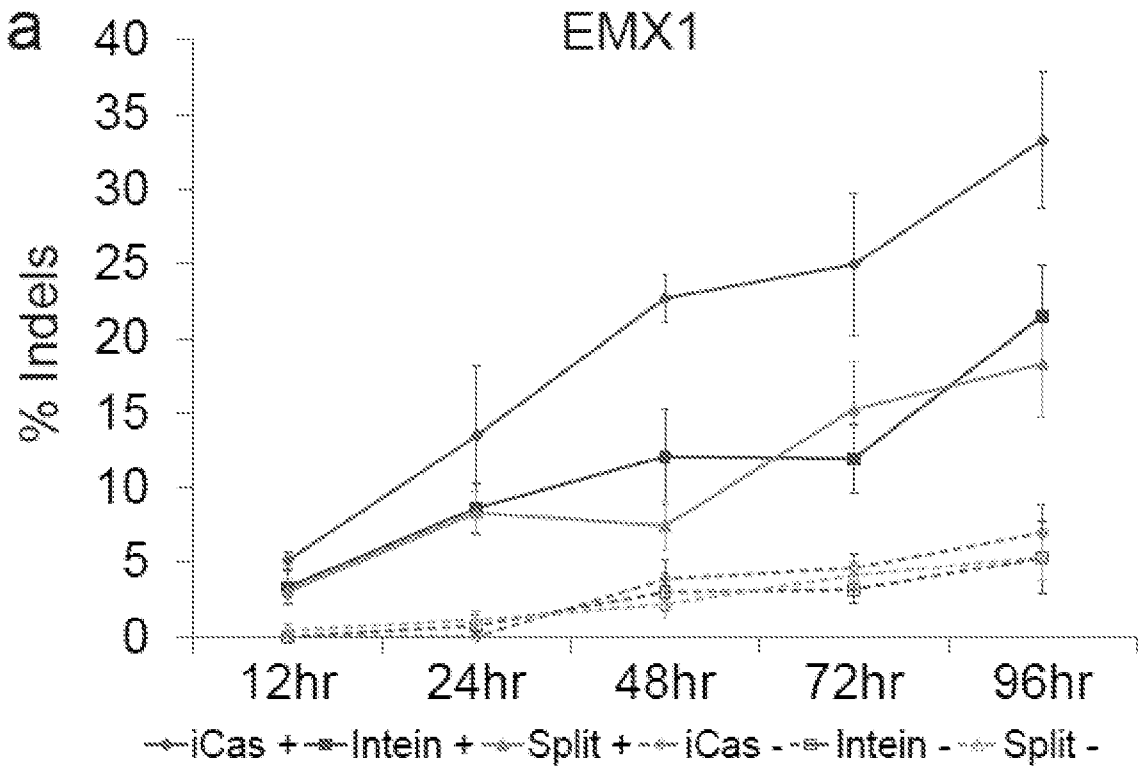


FIG. 5


a
EMX1
% Indels
40
35
30
25
20
15
10
5
0
12hr
24hr
48hr
72hr
96hr
iCas +
Intein +
Split +
iCas -
Intein -
Split -

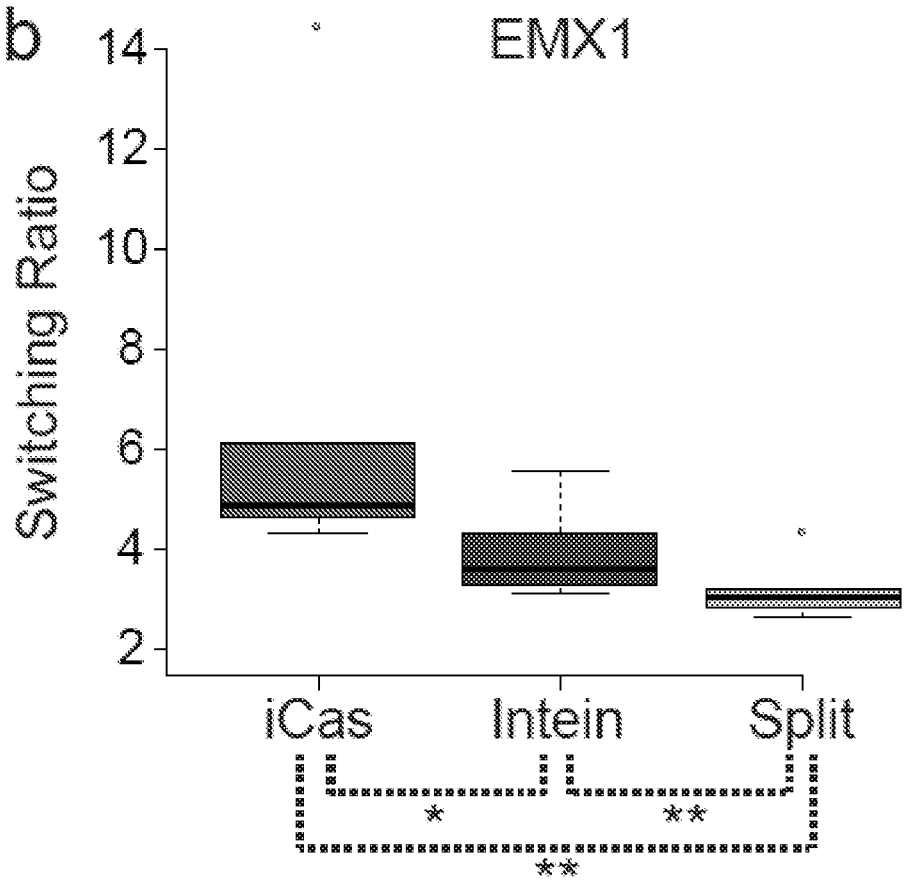

b
EMX1
Switching Ratio
14
12
10
8
6
4
2
iCas
Intein
Split
*
**
**

FIG. 5 continued

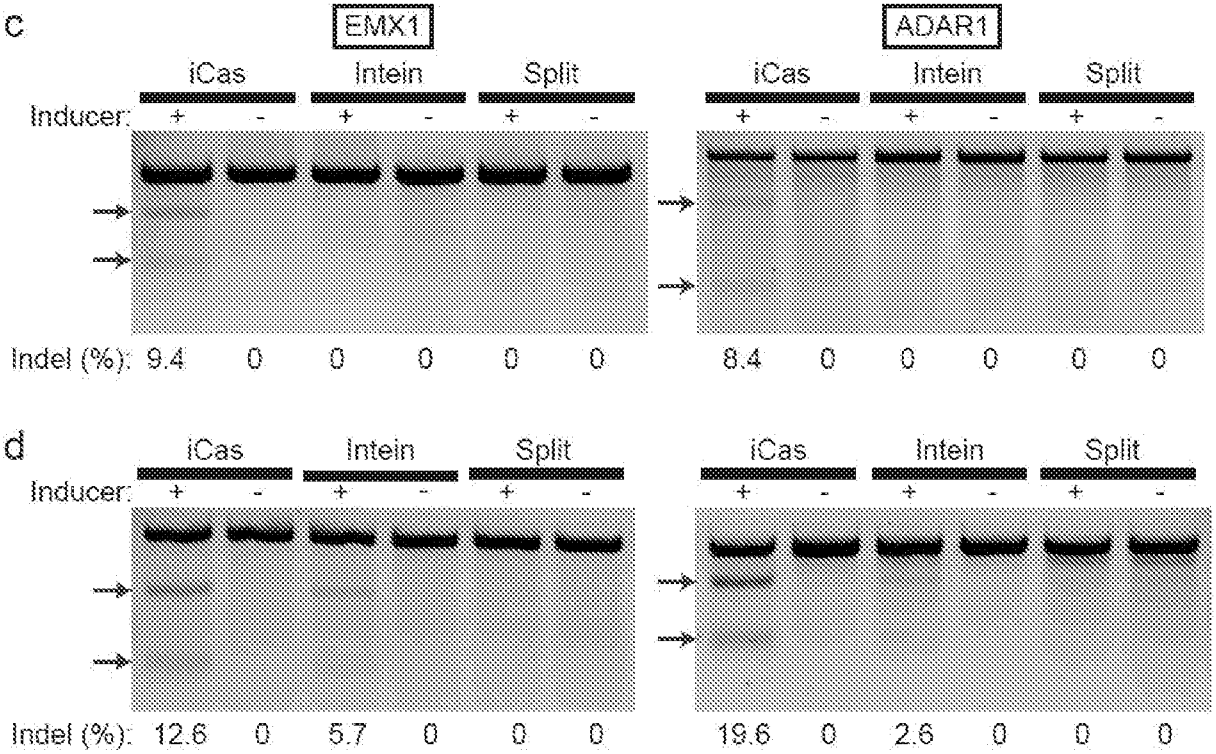


FIG. 6

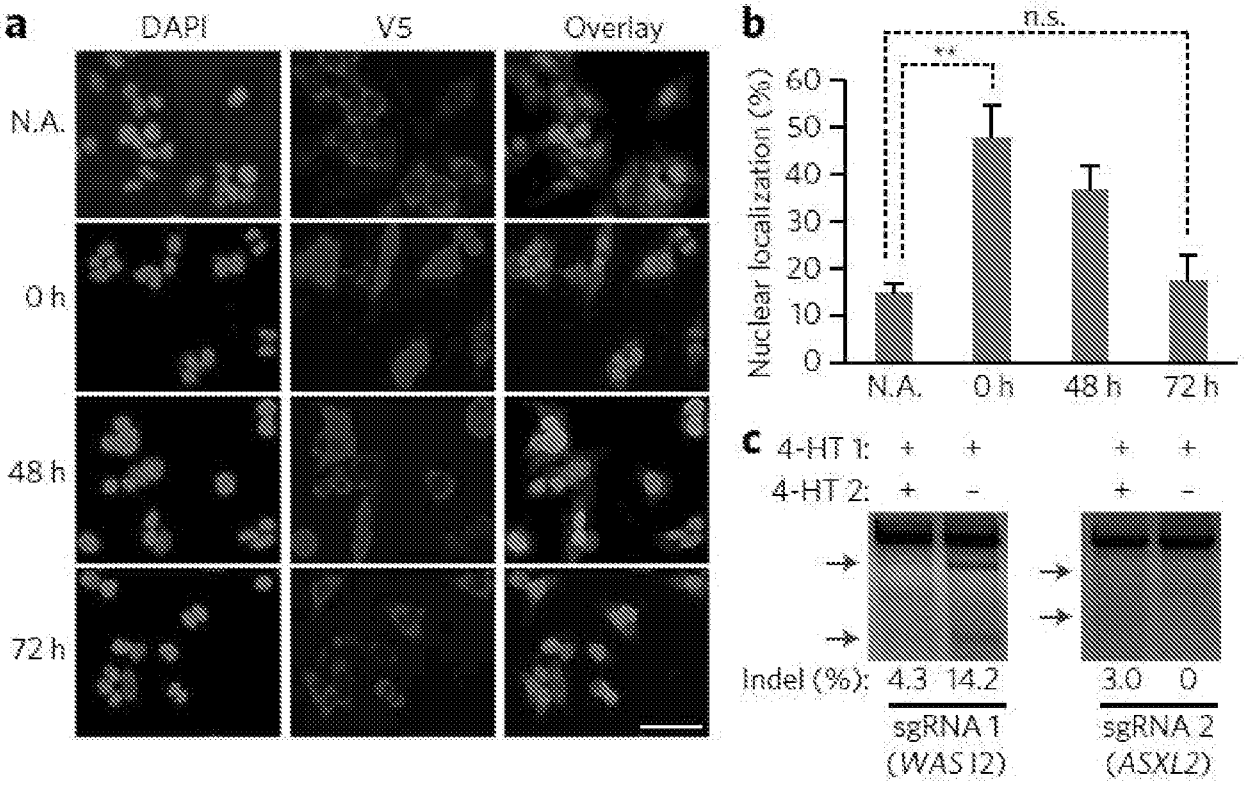


FIG. 7

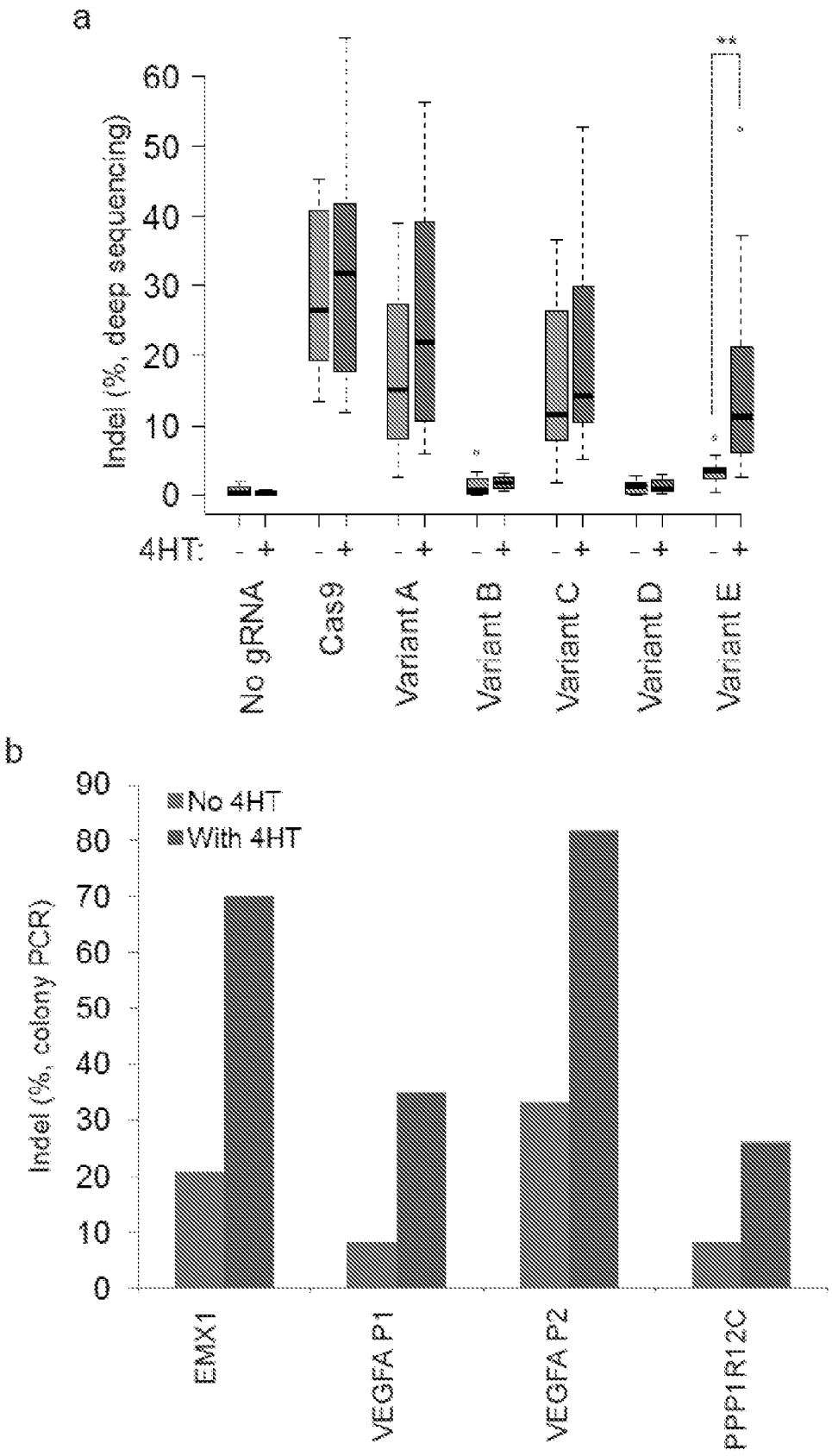

a
Indel (%, deep sequencing)
60
50
40
30
20
10
0
4HT:
- + - + - + - + - + - + - +
No gRNA
Cas9
Variant A
Variant B
Variant C
Variant D
Variant E
b
Indel (%, colony PCR)
90
80
70
60
50
40
30
20
10
0
No 4HT
With 4HT
EMX1
VEGFA P1
VEGFA P2
PPP1R12C

FIG. 8

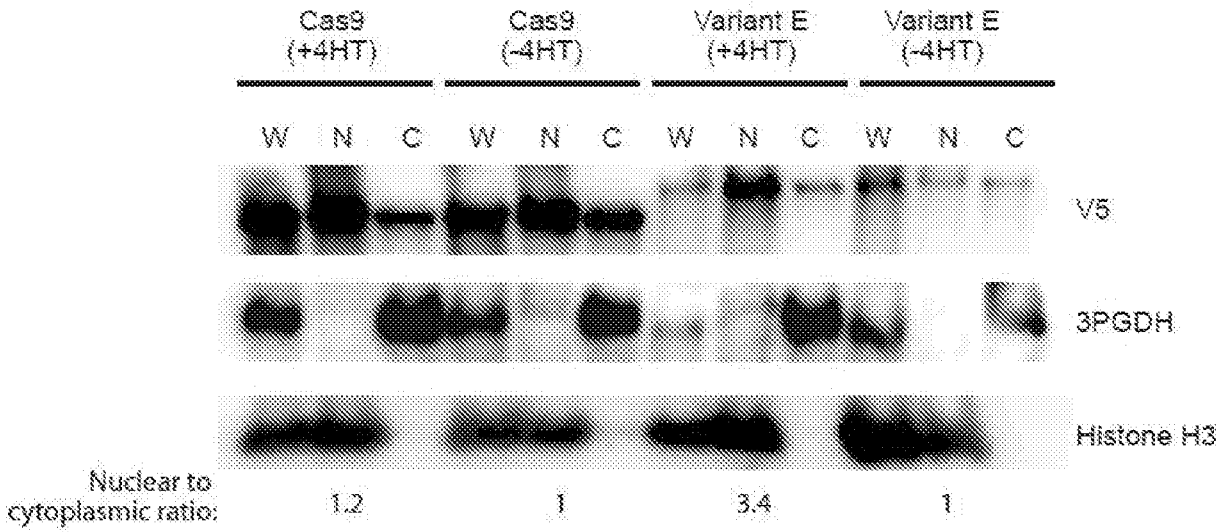

Cas9 (+4HT)
Cas9 (-4HT)
Variant E (+4HT)
Variant E (-4HT)
W N C W N C W N C W N C
V5
3PGDH
Histone H3
Nuclear to cytoplasmic ratio:
1.2
1
3.4
1

FIG. 9

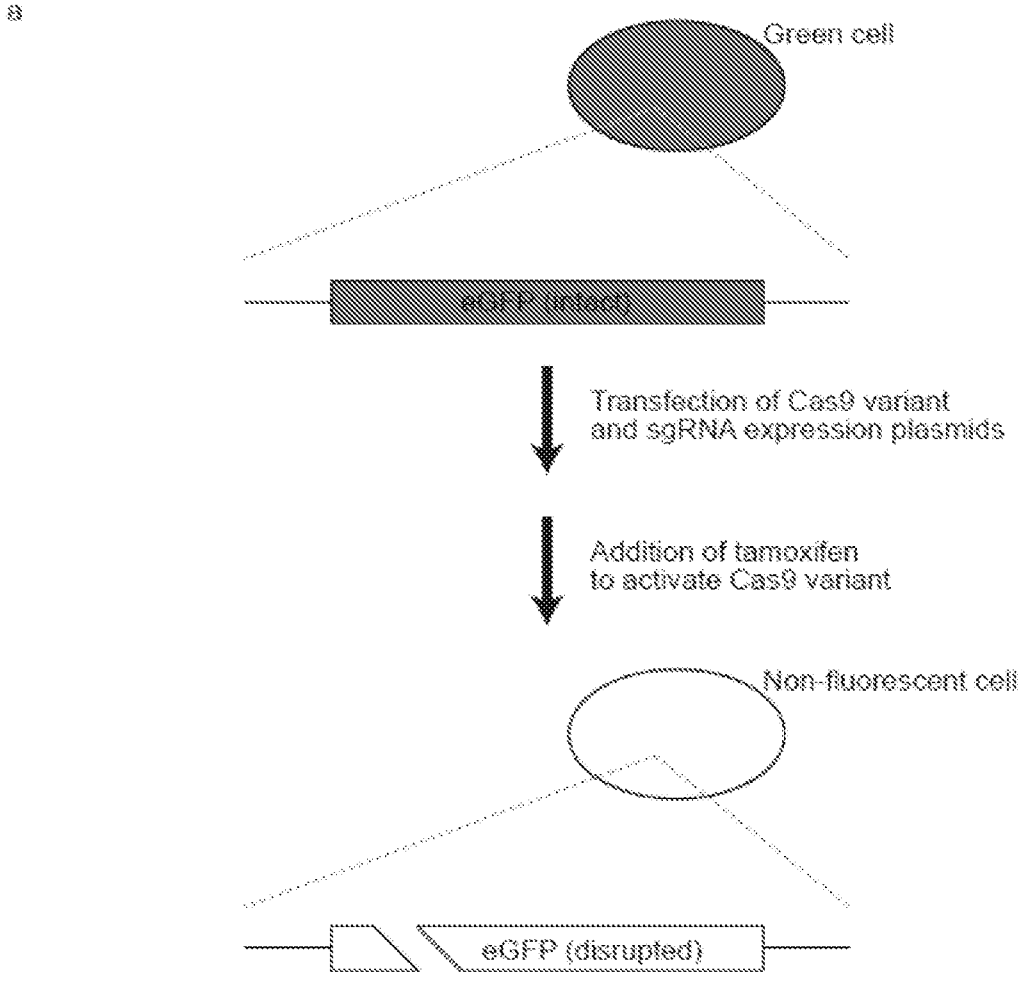

a
Green cell
Transfection of Cas9 variant and sgRNA expression plasmids
Addition of tamoxifen to activate Cas9 variant
Non-fluorescent cell
eGFP (disrupted)

FIG. 9 continued

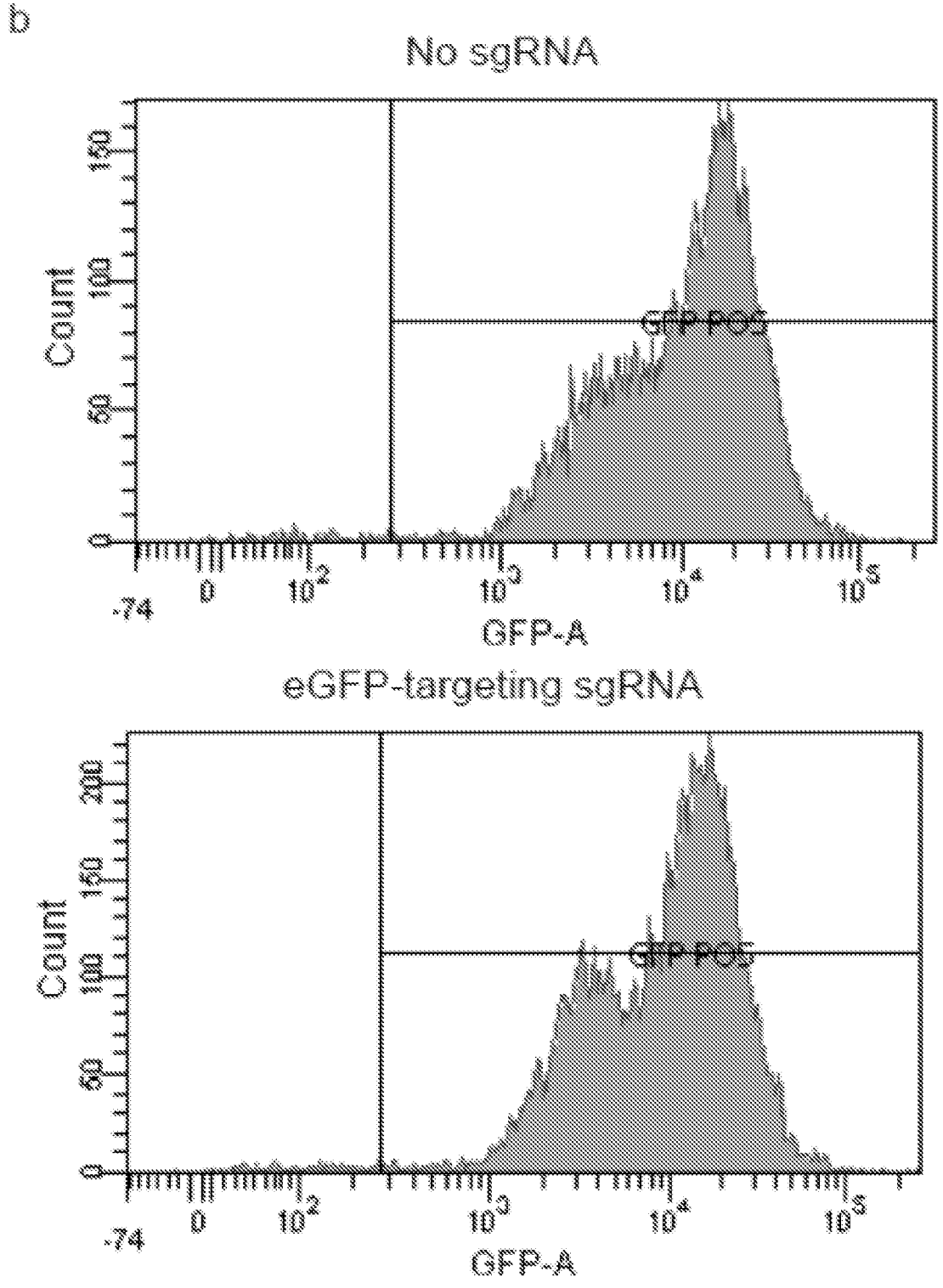


FIG. 10

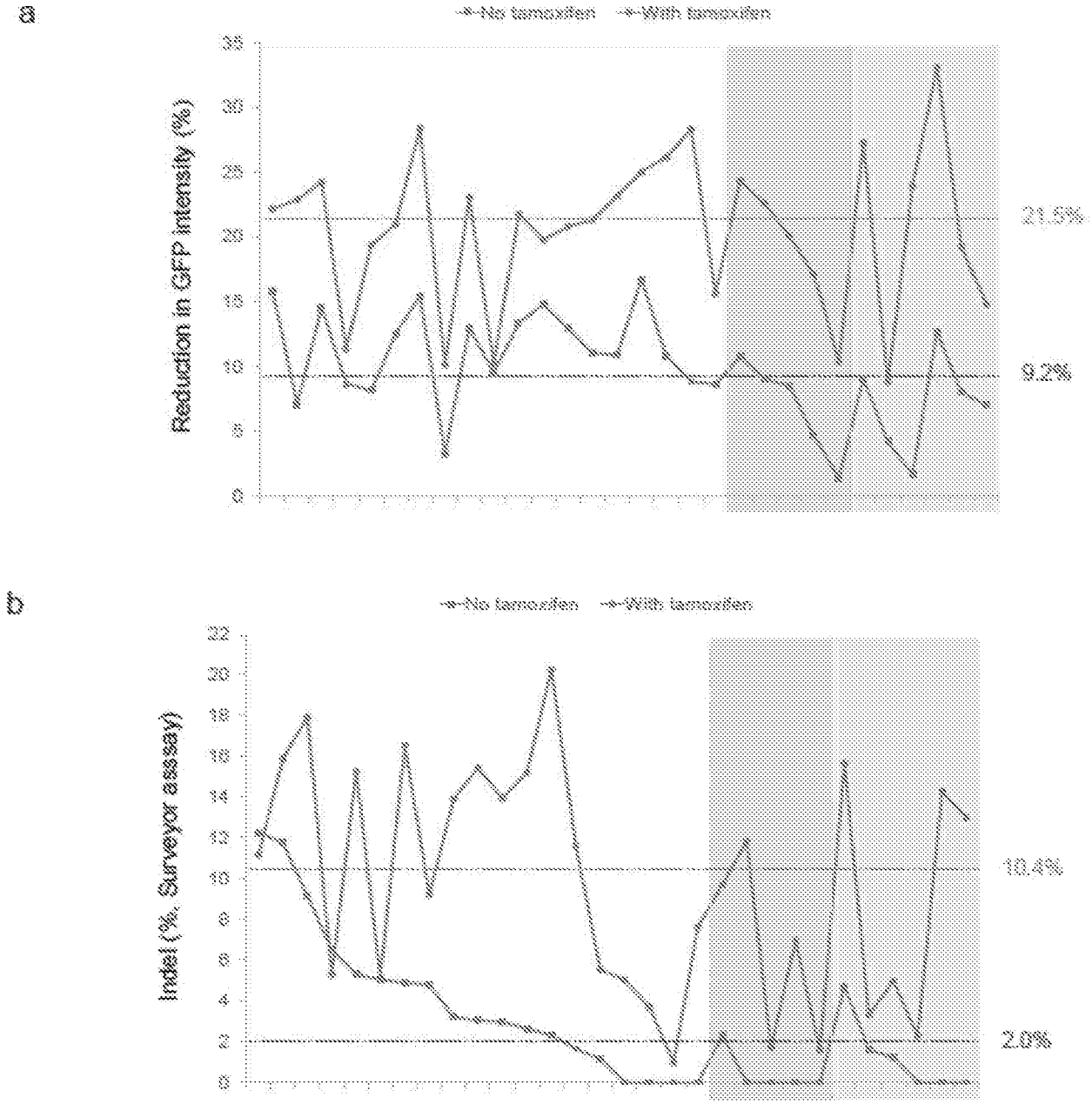

a
No tamoxifen
With tamoxifen
Reduction in GFP intensity (%)
21.6%
9.2%
b
No tamoxifen
With tamoxifen
Indel (%, Surveyor assay)
10.4%
2.0%

FIG. 10 continued

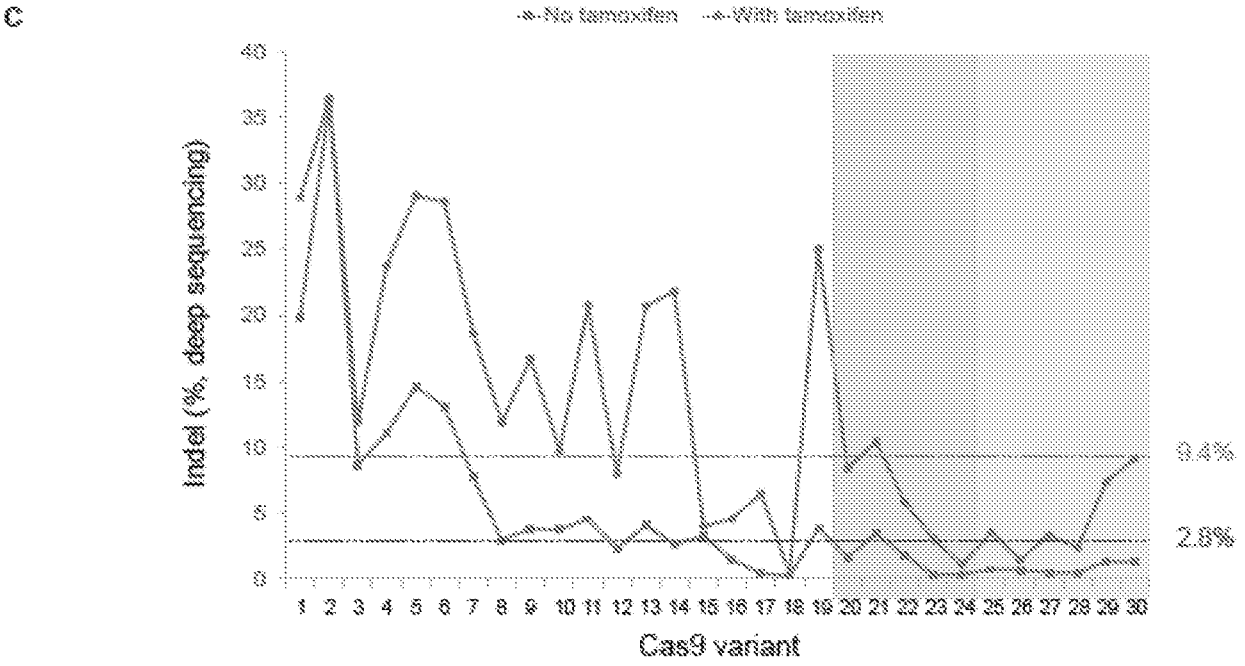


FIG. 11

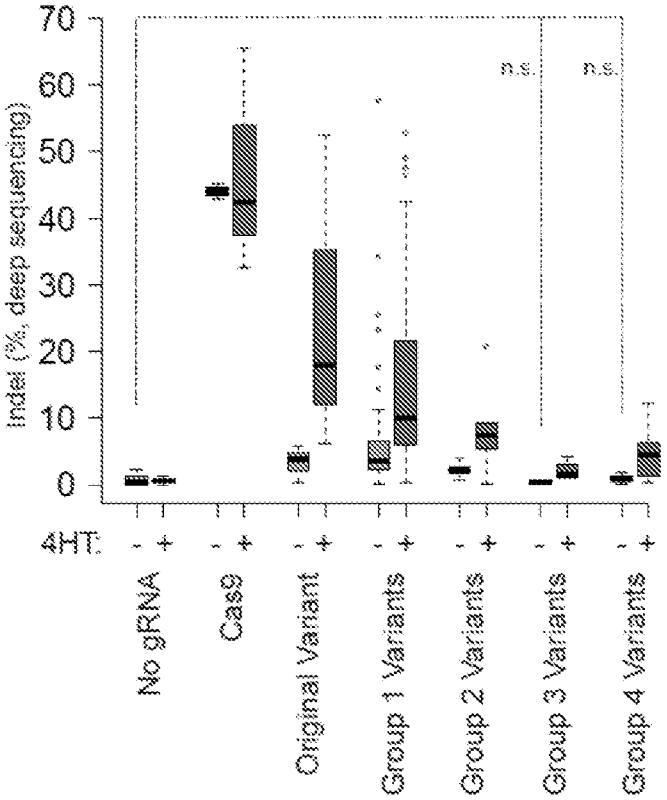


FIG. 12

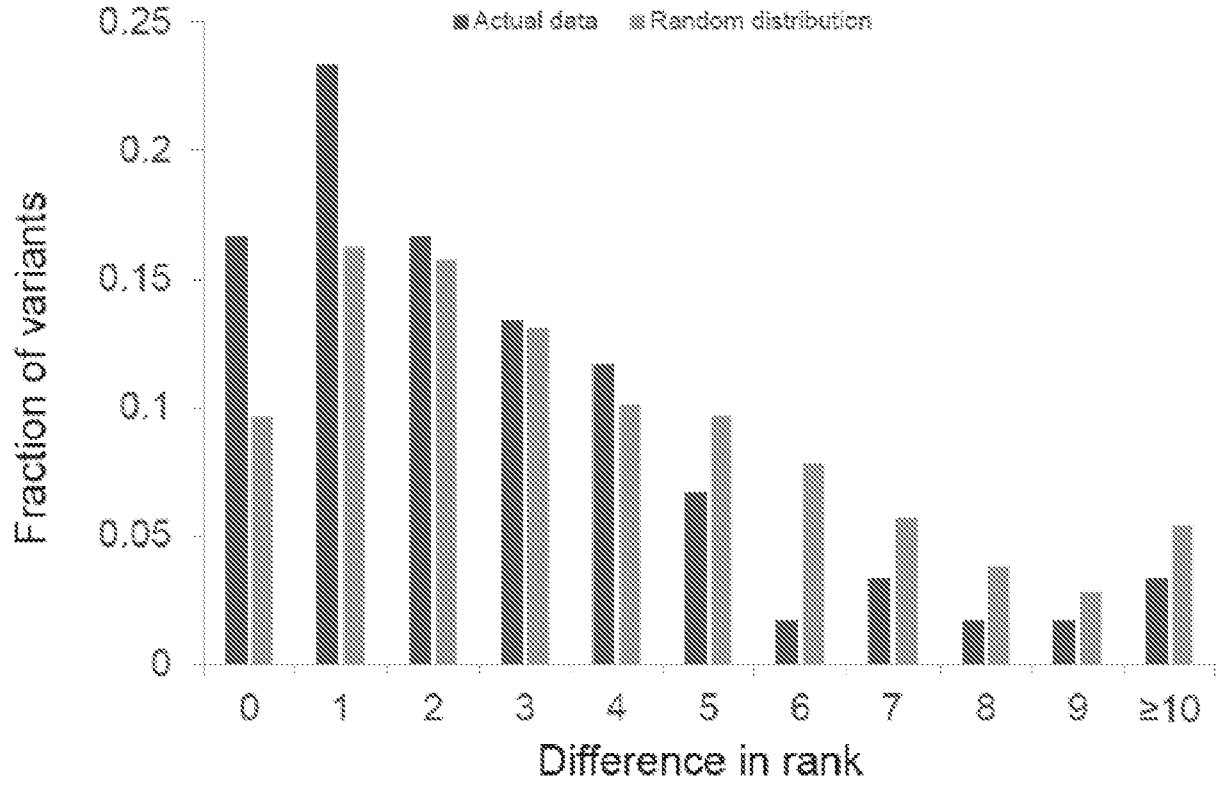

Actual data
Random distribution
Fraction of variants
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
4
5
6
7
8
9
≥10
Difference in rank

FIG. 13

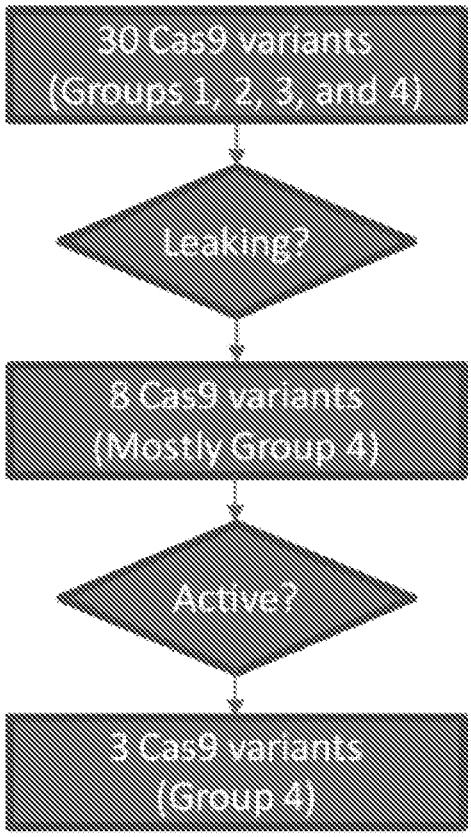

30 Cas9 variants
(Groups 1, 2, 3, and 4)
Leaking?
8 Cas9 variants
(Mostly Group 4)
Active?
3 Cas9 variants
(Group 4)

FIG. 14

a

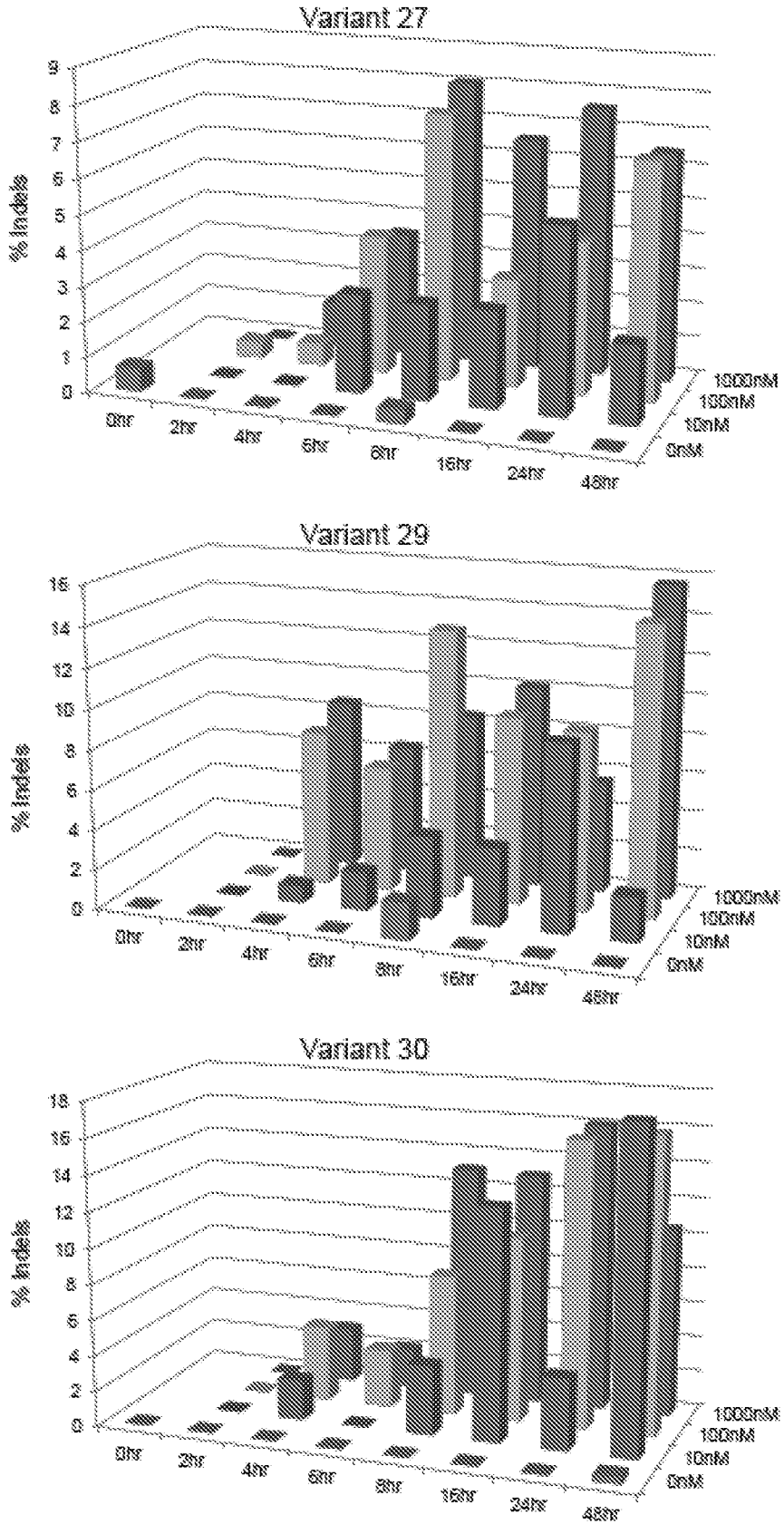

Variant 27
% Indels
0
1
2
3
4
5
6
7
8
9
0hr
2hr
4hr
6hr
8hr
16hr
24hr
48hr
1000nM
100nM
10nM
0nM
Variant 29
% Indels
0
2
4
6
8
10
12
14
16
0hr
2hr
4hr
6hr
8hr
16hr
24hr
48hr
1000nM
100nM
10nM
0nM
Variant 30
% Indels
0
2
4
6
8
10
12
14
16
18
0hr
2hr
4hr
6hr
8hr
16hr
24hr
48hr
1000nM
100nM
10nM
0nM

FIG. 14 continued

b

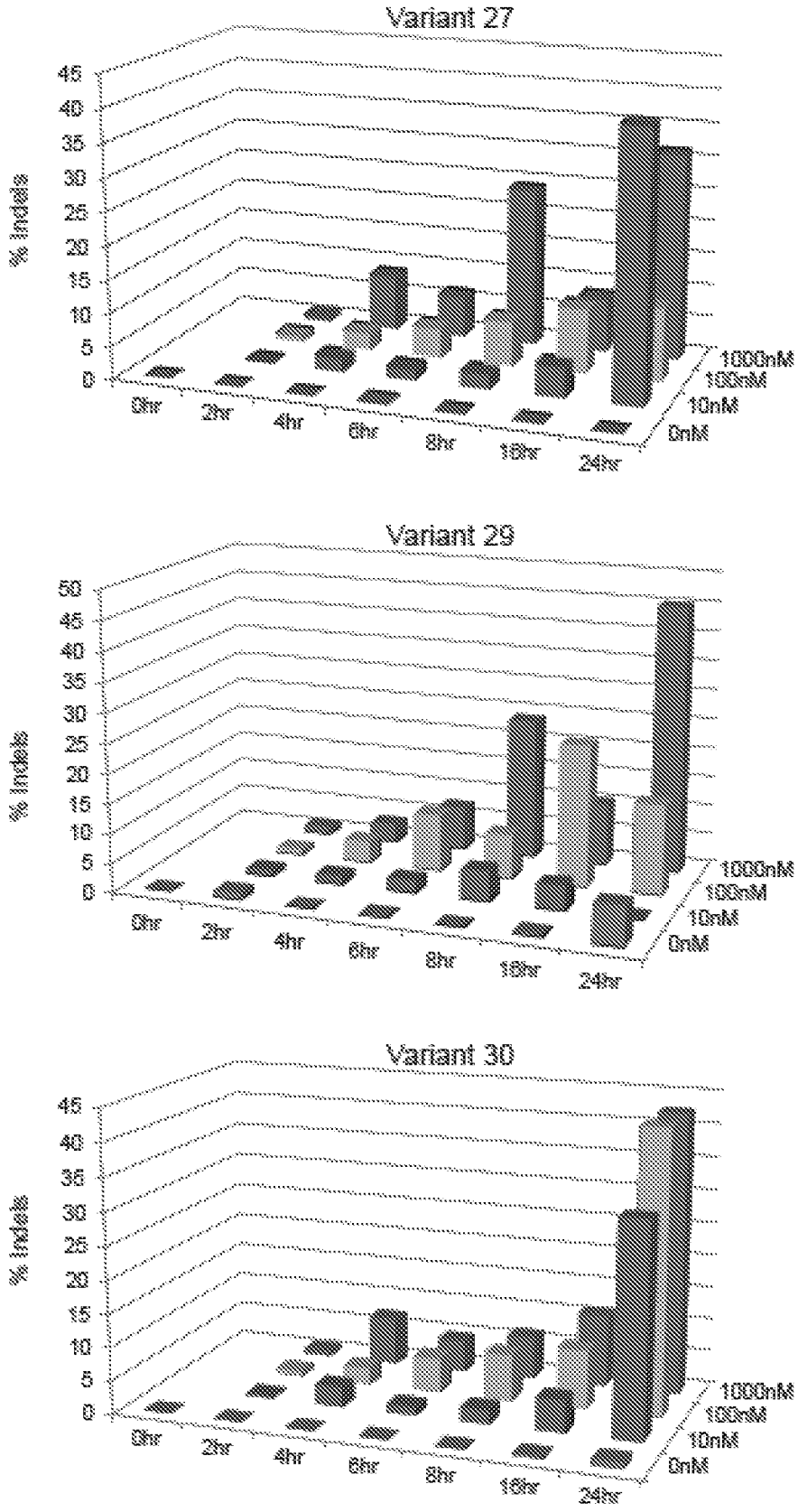


FIG. 15

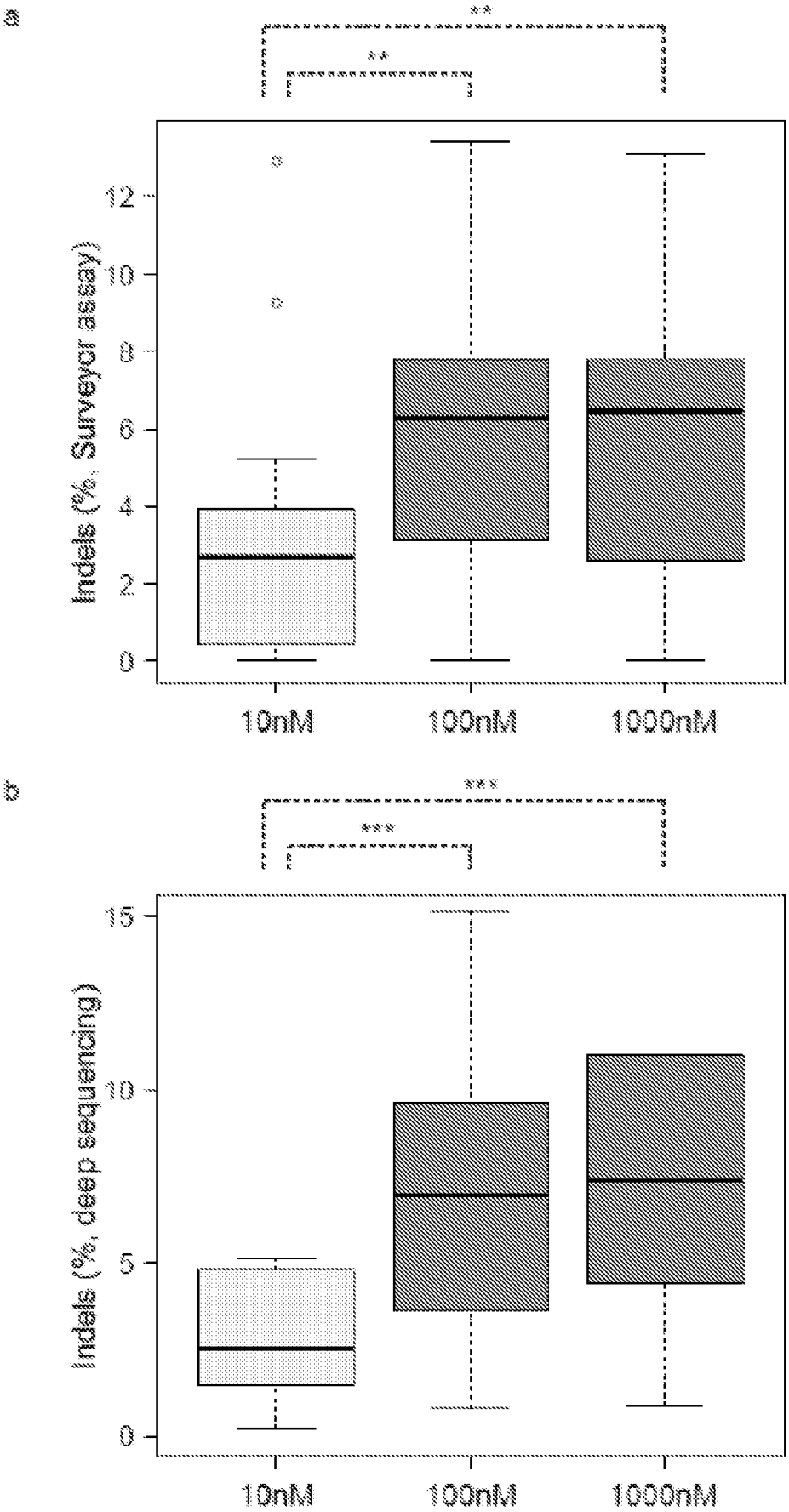

a
**
**
Indels (%, Surveyor assay)
0
2
4
6
8
10
12
10nM
100nM
1000nM
b
***
***
Indels (%, deep sequencing)
0
5
10
15
10nM
100nM
1000nM

FIG: 16

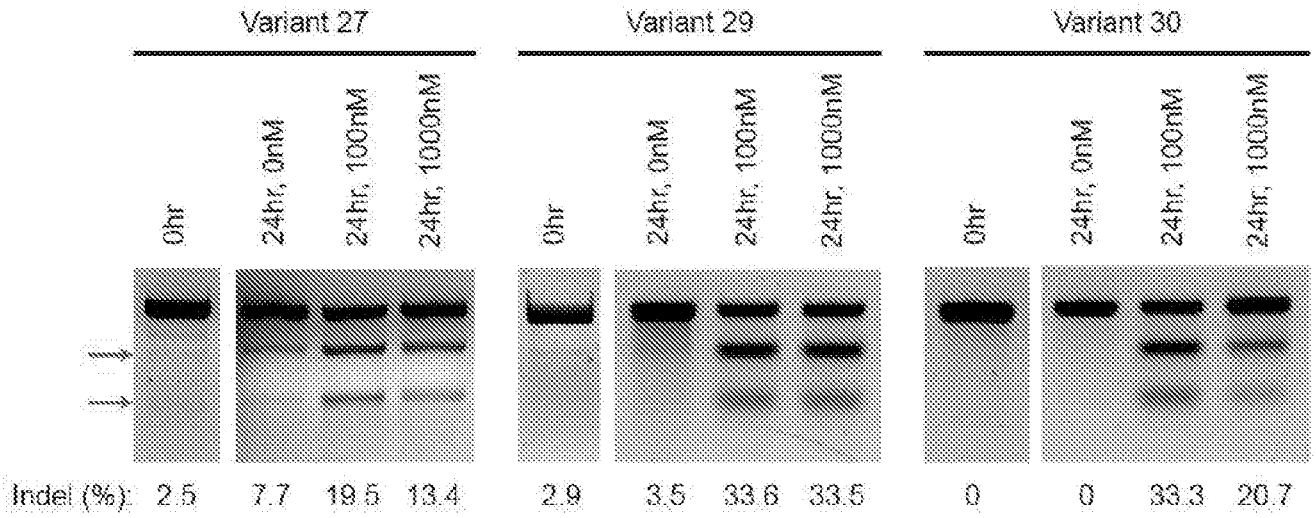


FIG. 17

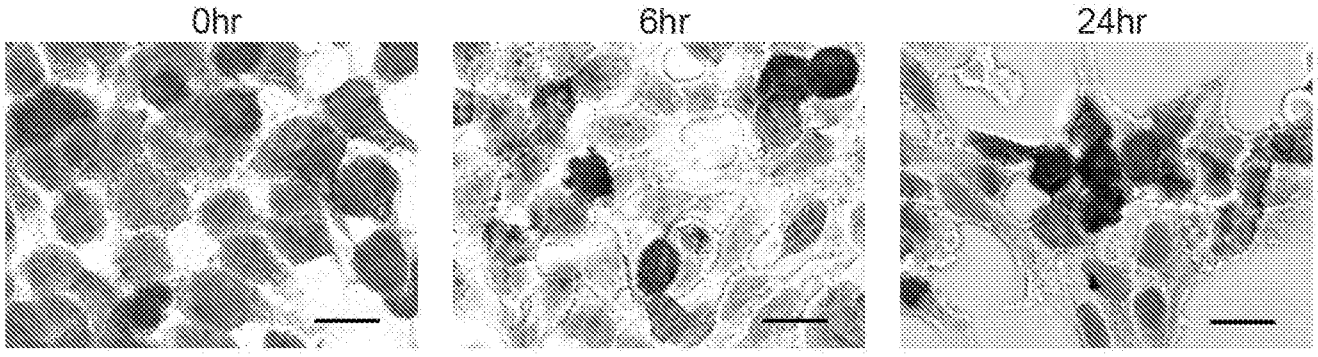


FIG. 18

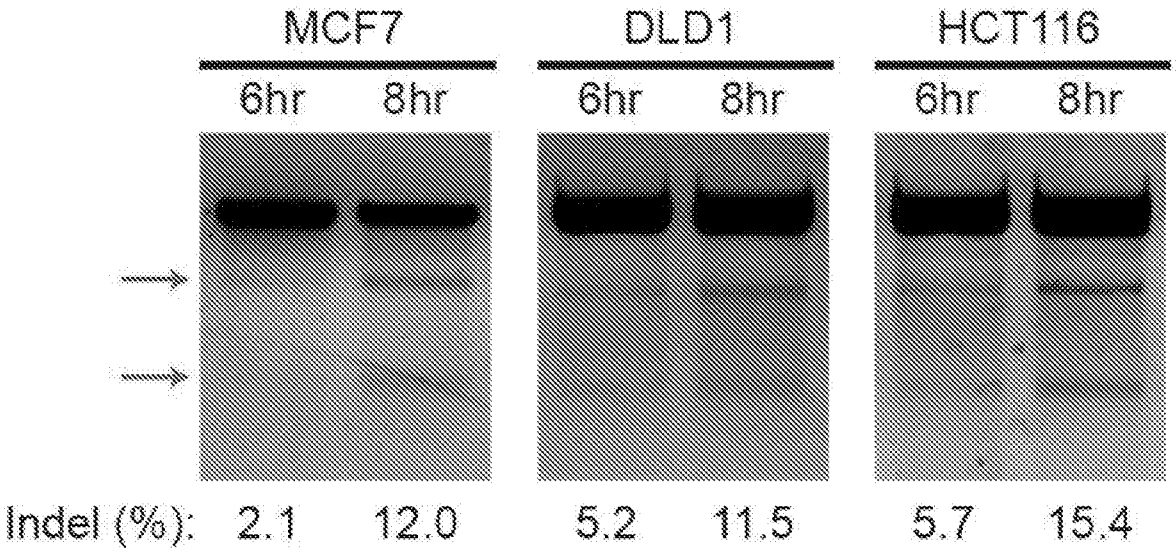


FIG. 19

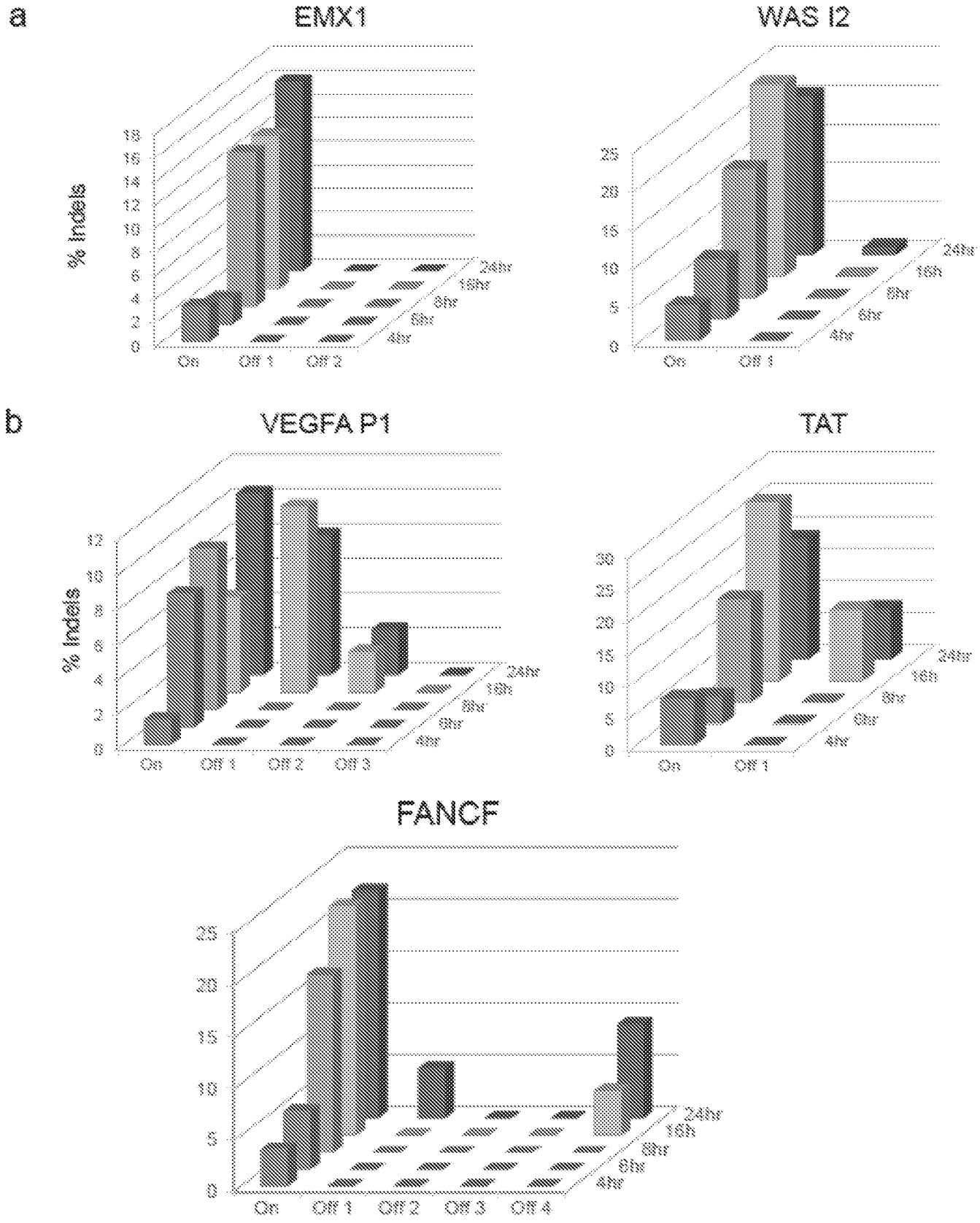

a
EMX1
% Indels
0
2
4
6
8
10
12
14
16
18
On
Off 1
Off 2
24hr
16hr
8hr
6hr
4hr
WAS I2
0
5
10
15
20
25
On
Off 1
24hr
16h
8hr
6hr
4hr
b
VEGFA P1
% Indels
0
2
4
6
8
10
12
On
Off 1
Off 2
Off 3
24hr
16h
8hr
6hr
4hr
TAT
0
5
10
15
20
25
30
On
Off 1
24hr
16h
8hr
6hr
4hr
FANCF
0
5
10
15
20
25
On
Off 1
Off 2
Off 3
Off 4
24hr
16h
8hr
6hr
4hr

FIG. 19 continued

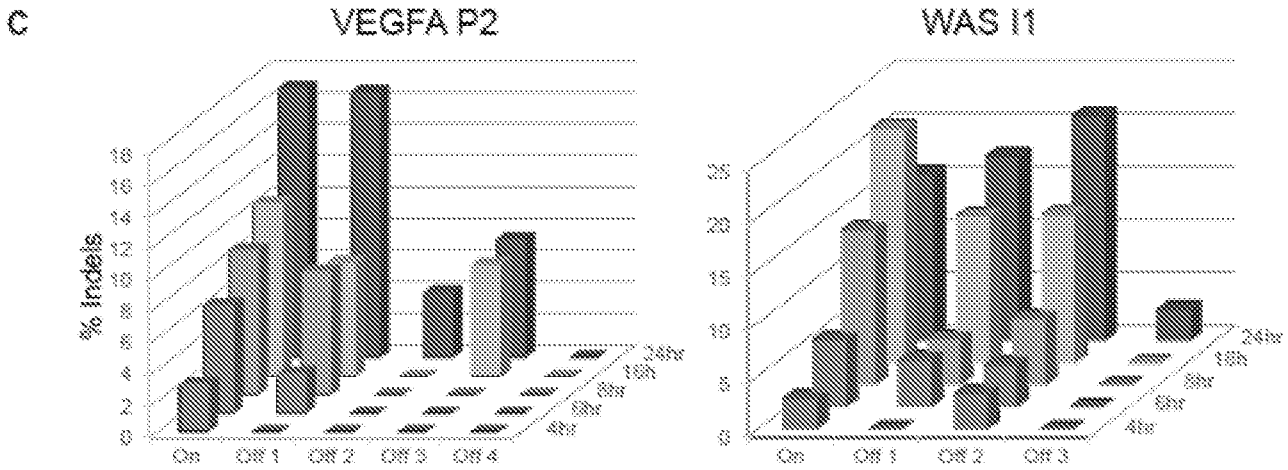


FIG. 20

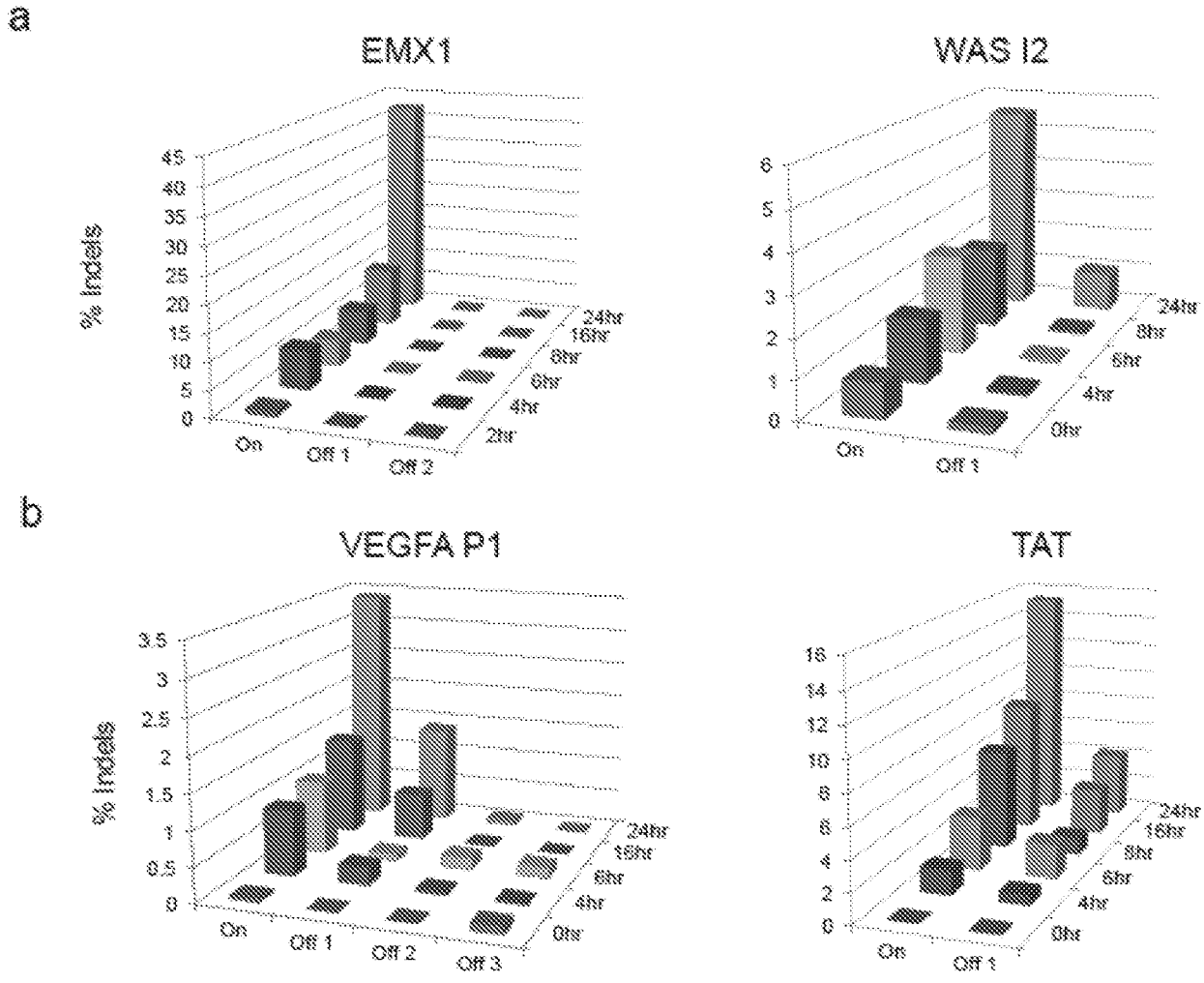
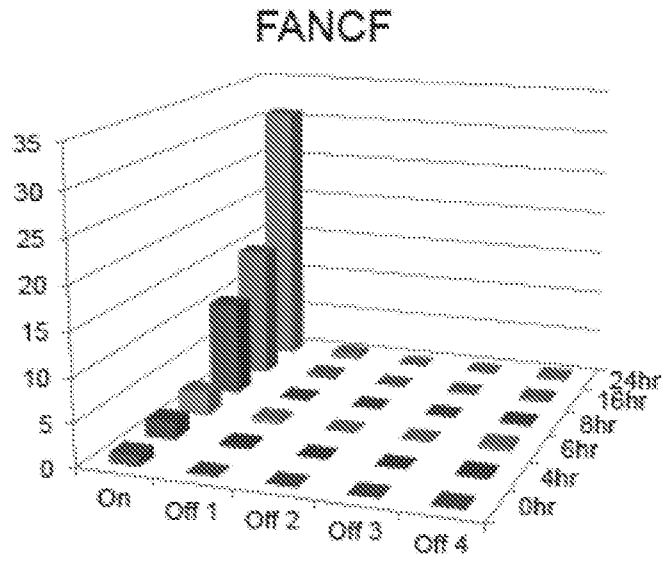


FIG. 20 continued



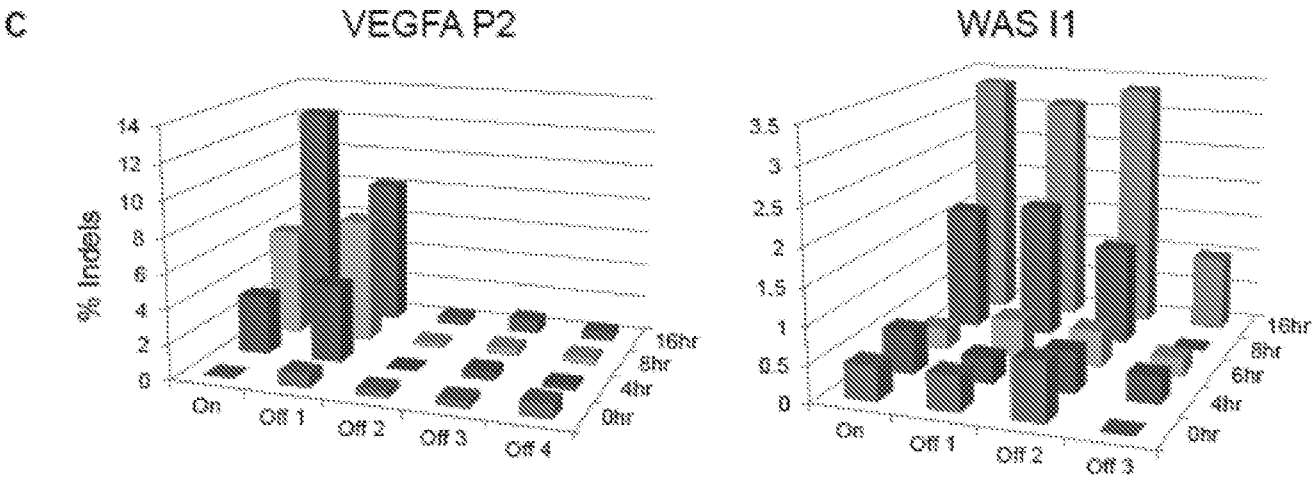


FIG. 21

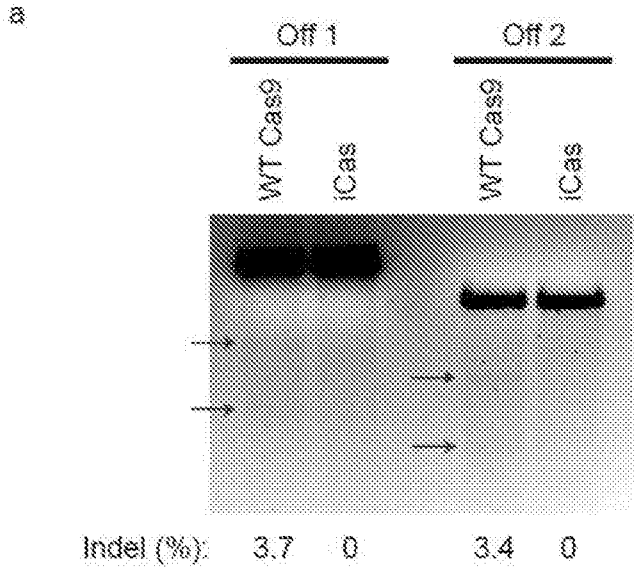


FIG. 21 continued

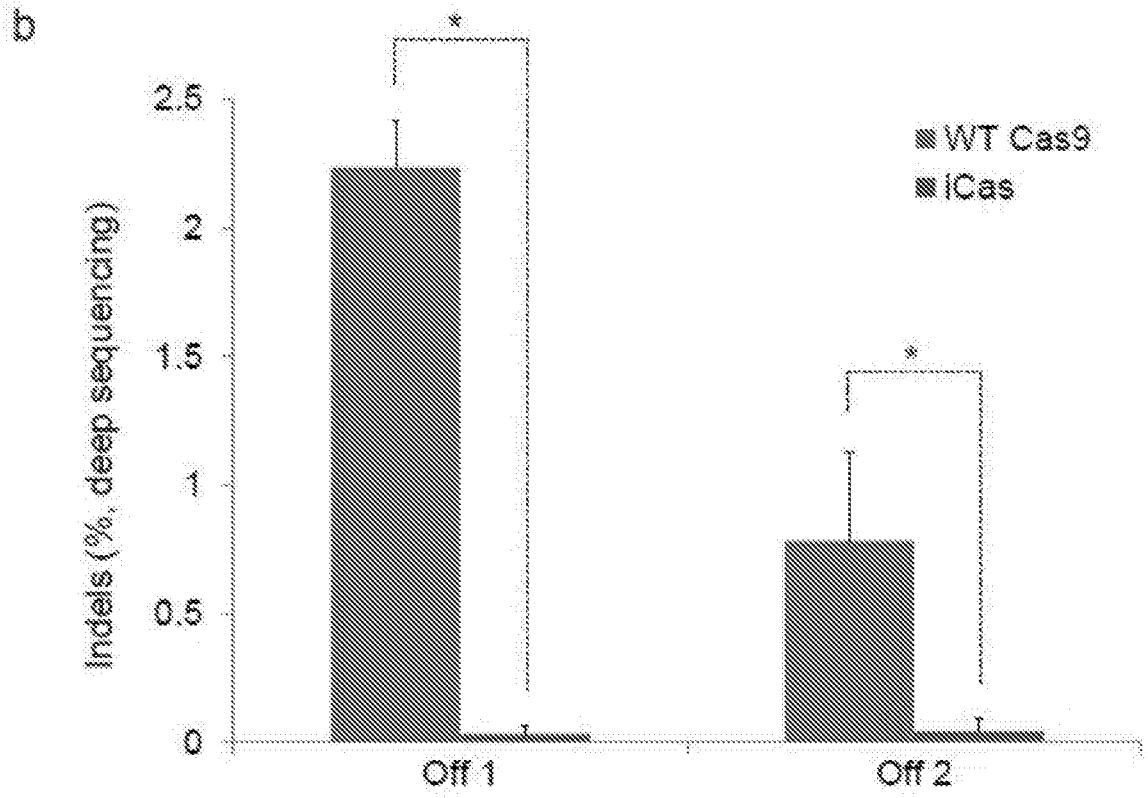


FIG. 22

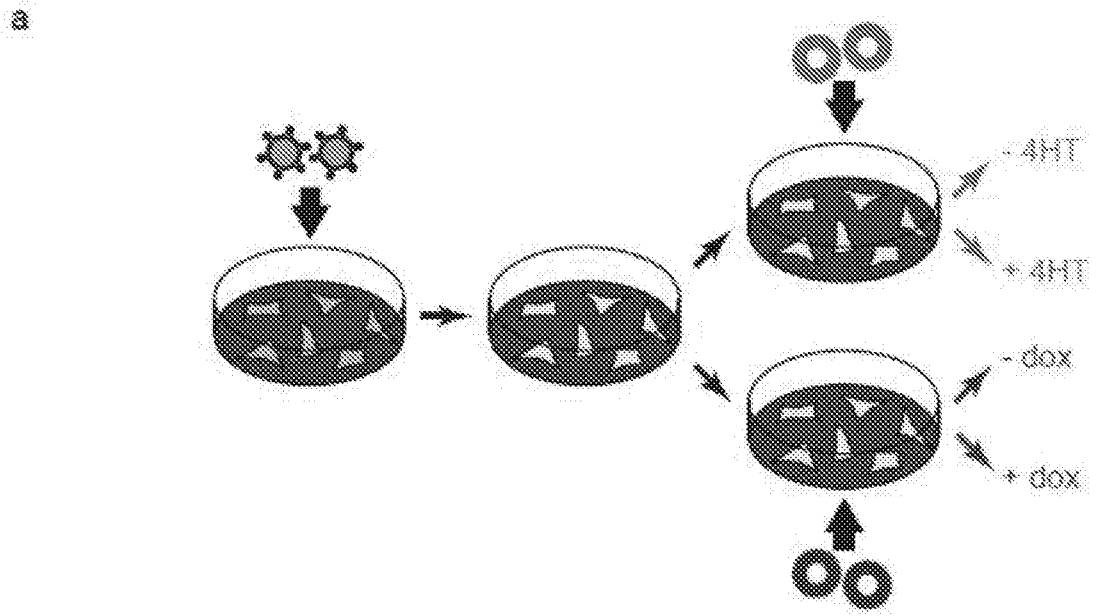


FIG. 22 continued

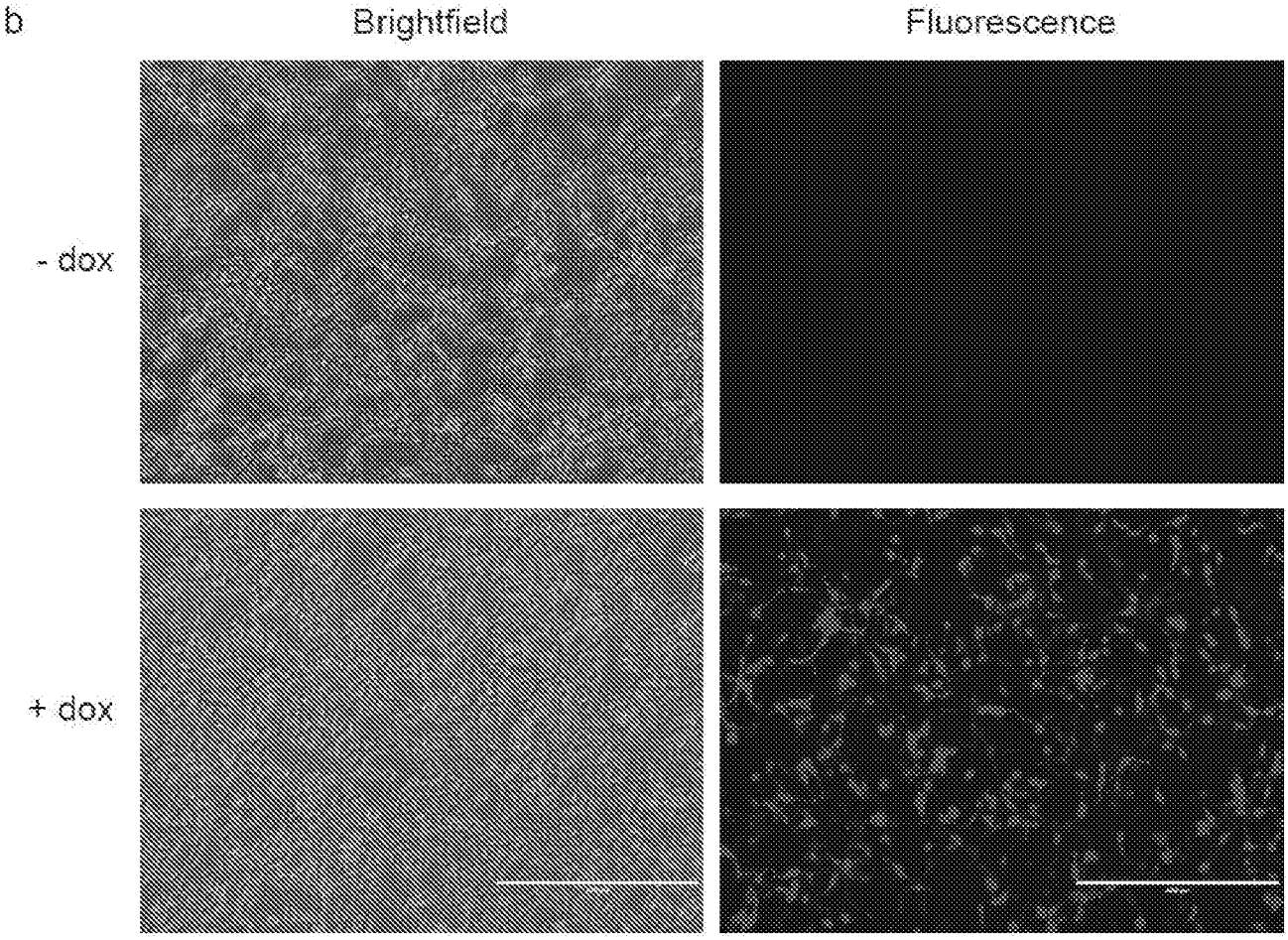


FIG. 23

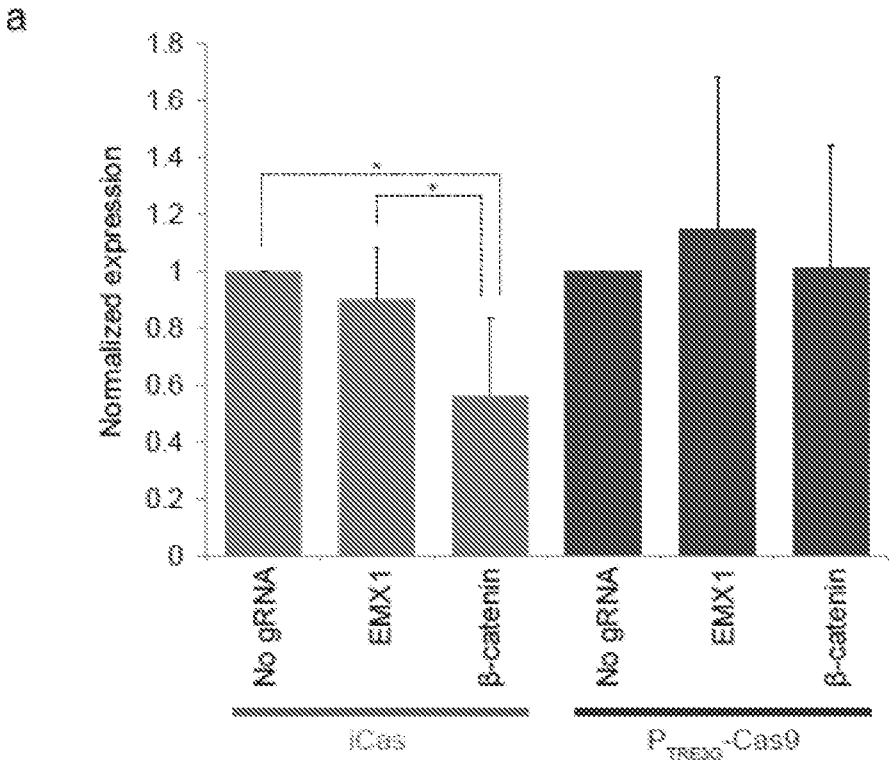


FIG. 23 continued

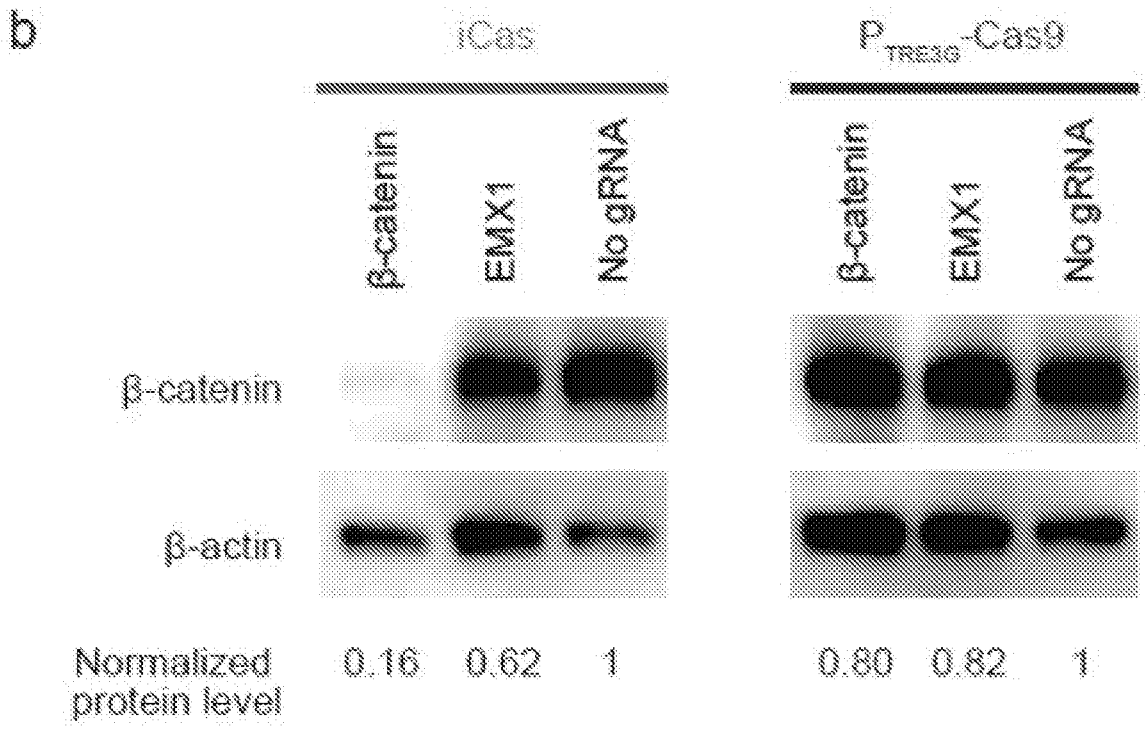


FIG. 24

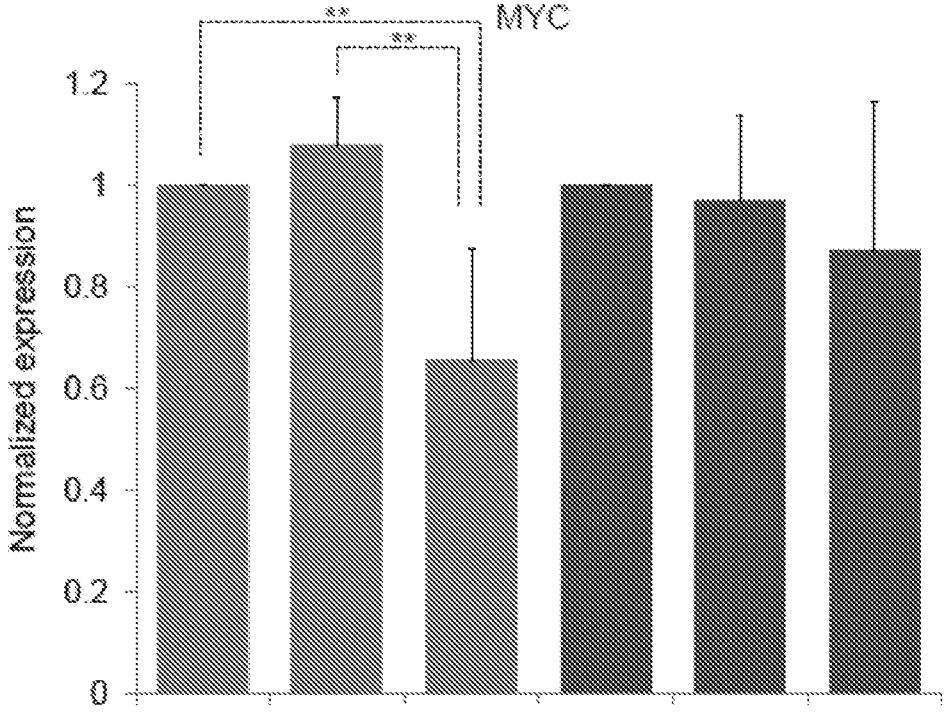


FIG. 24 continued

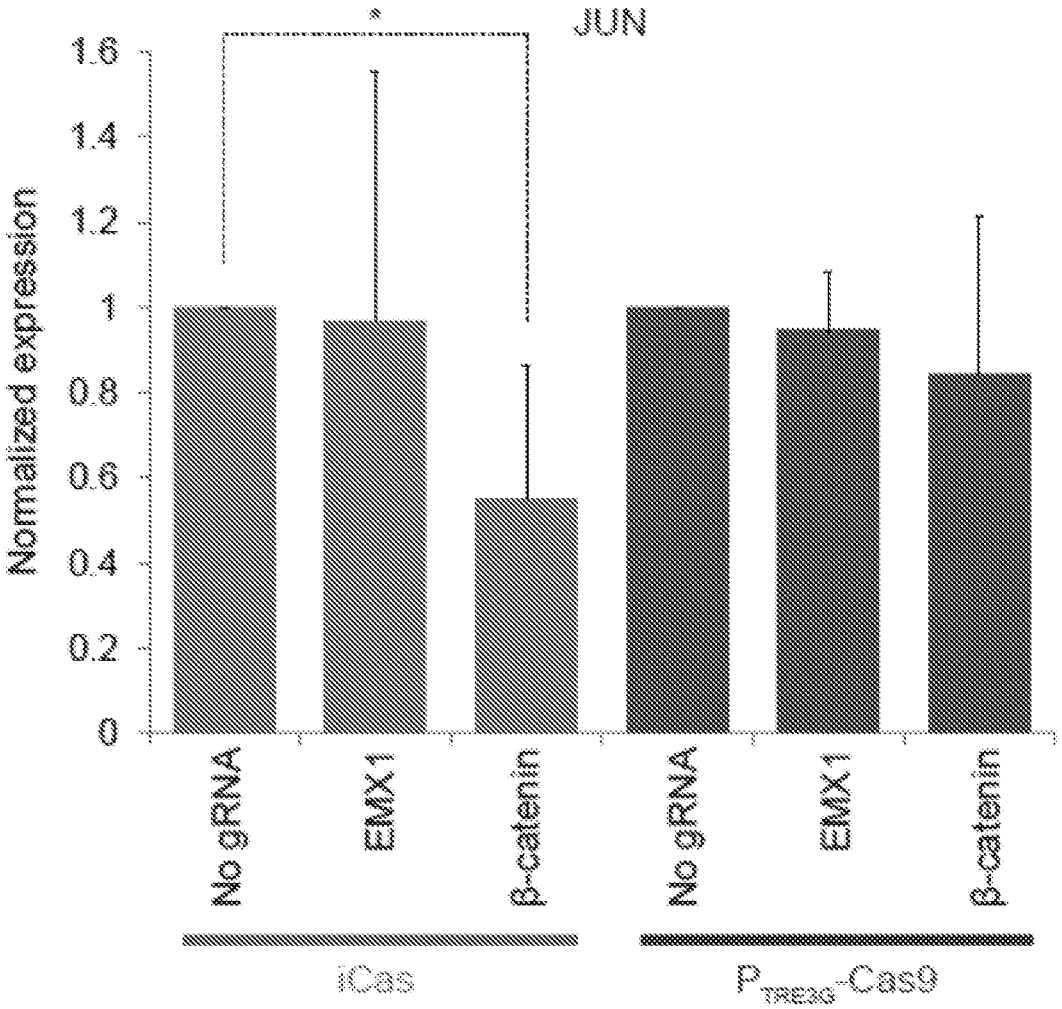


FIG. 25

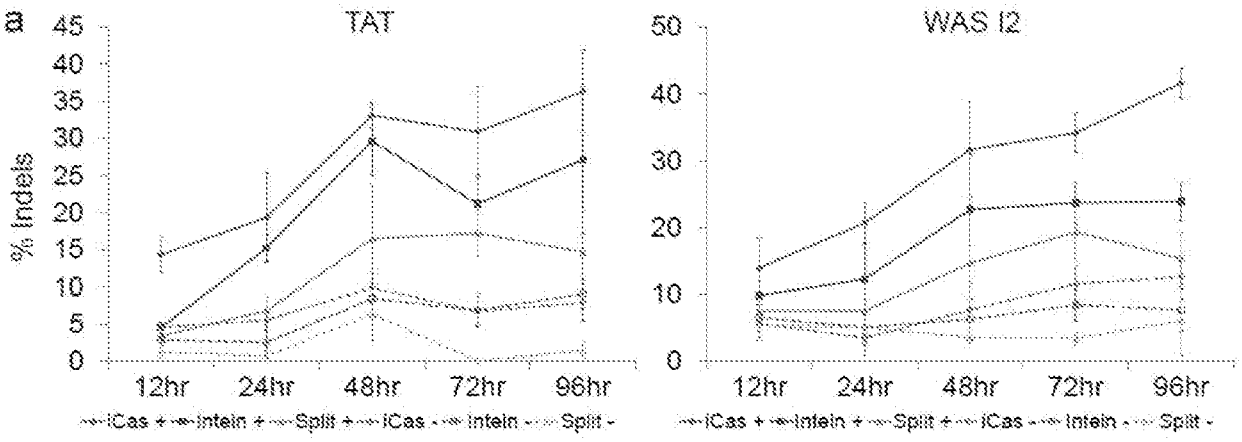


FIG. 25 continued

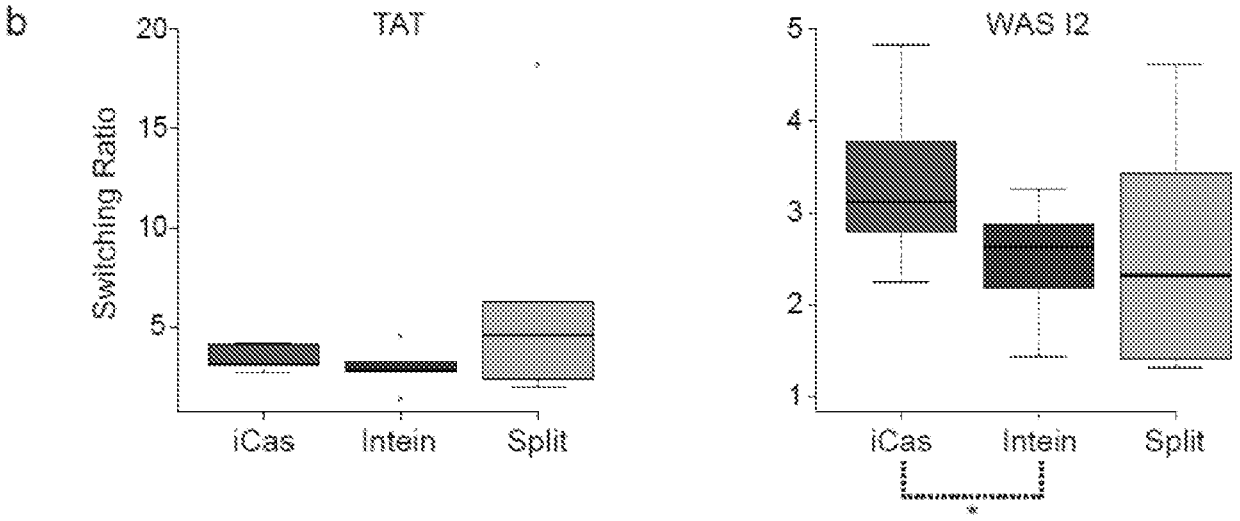


FIG. 26

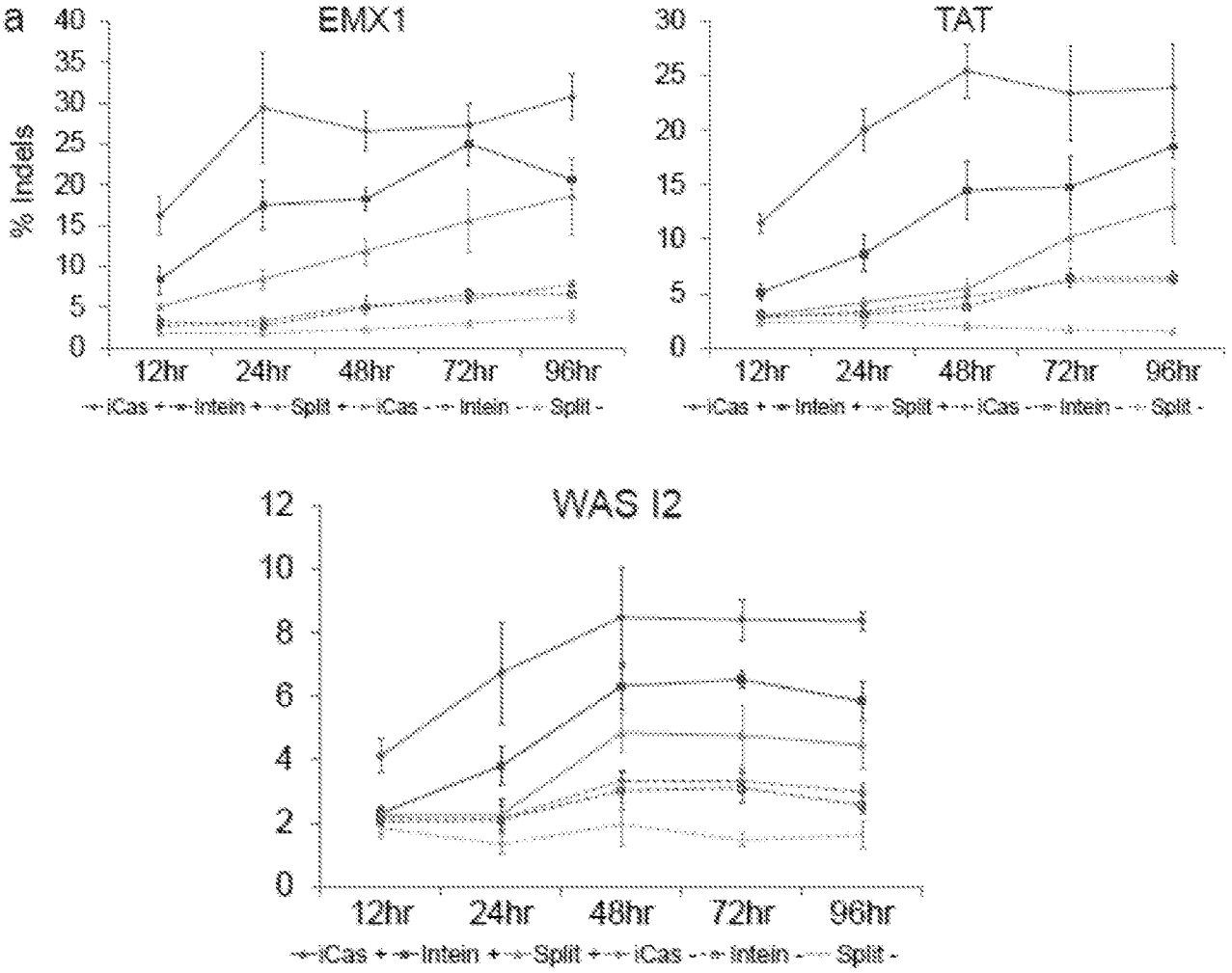


FIG. 26 continued

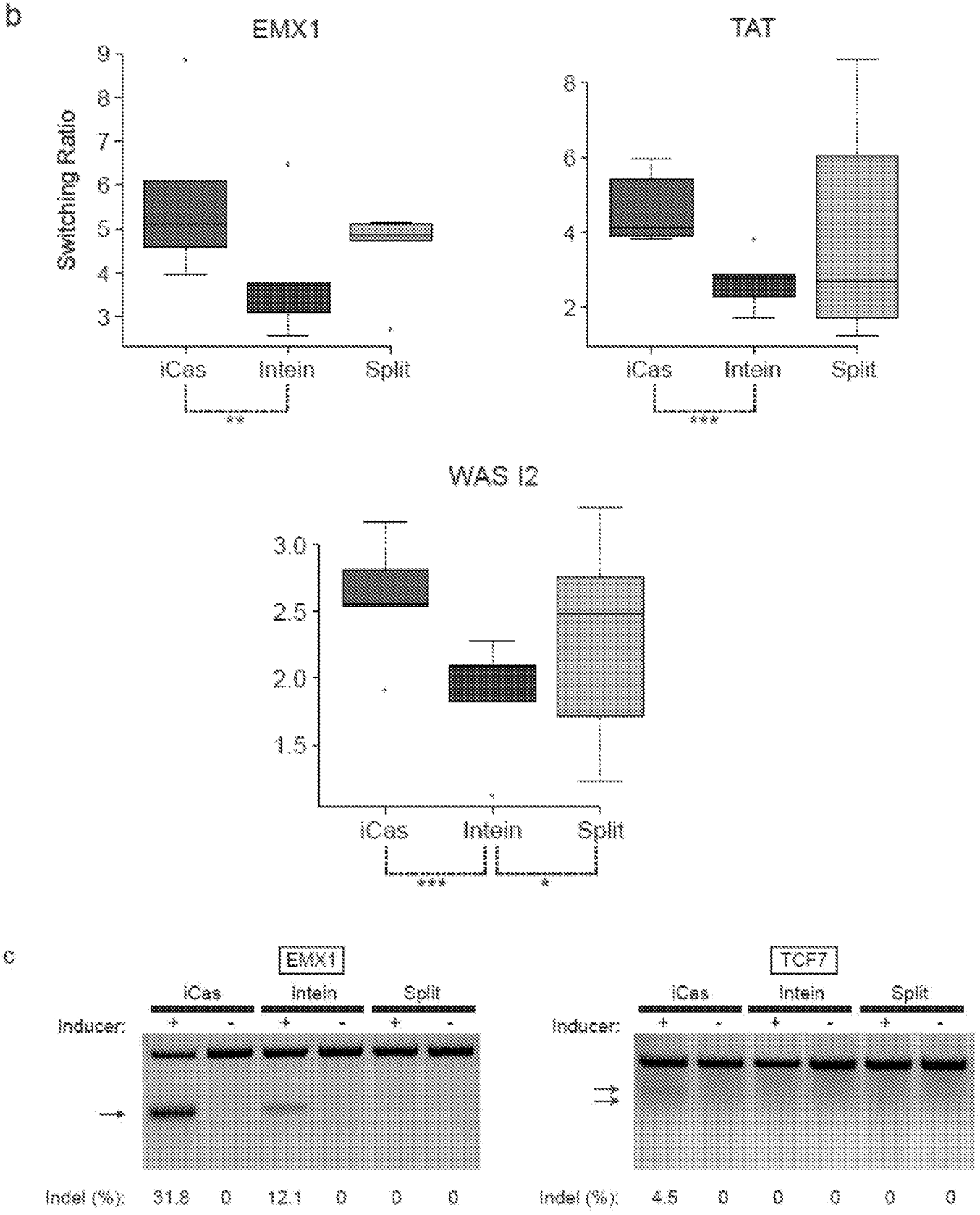


FIG. 27

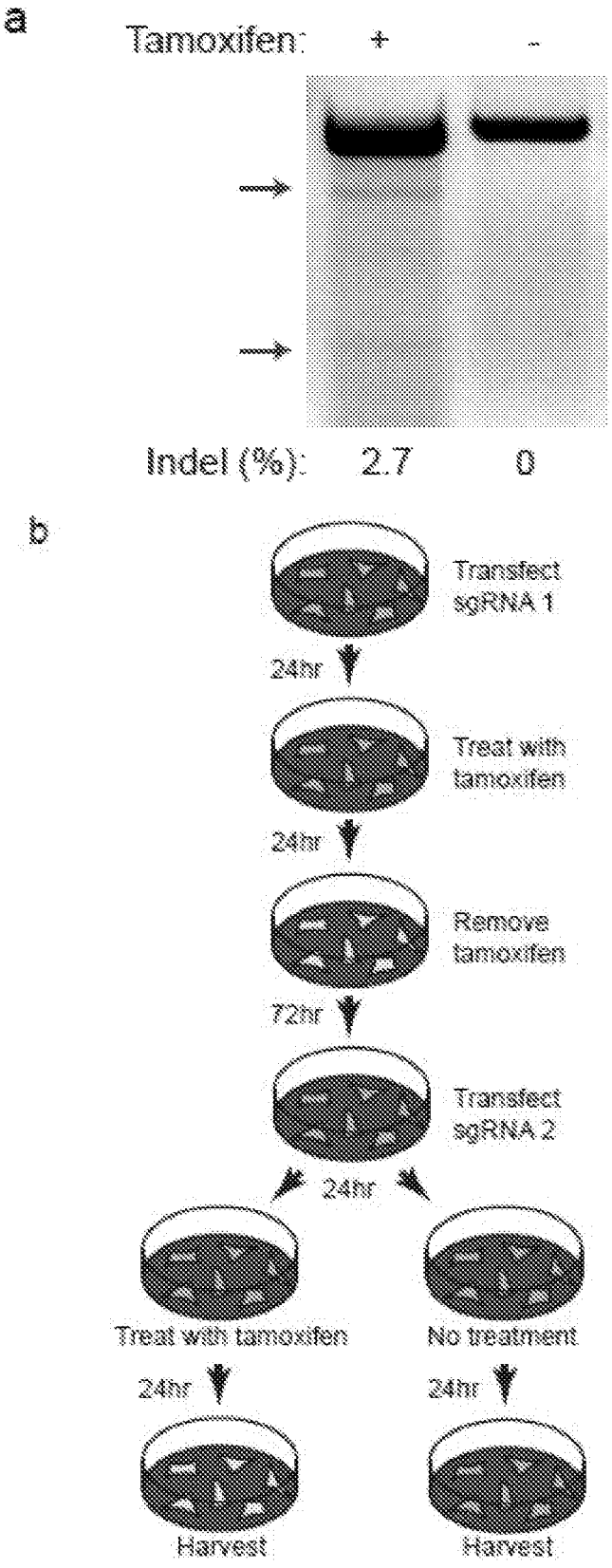

a
Tamoxifen: + -
Indel (%): 2.7 0
b
Transfect sgRNA 1
24hr
Treat with tamoxifen
24hr
Remove tamoxifen
72hr
Transfect sgRNA 2
24hr
Treat with tamoxifen
No treatment
24hr
24hr
Harvest
Harvest

FIG. 28

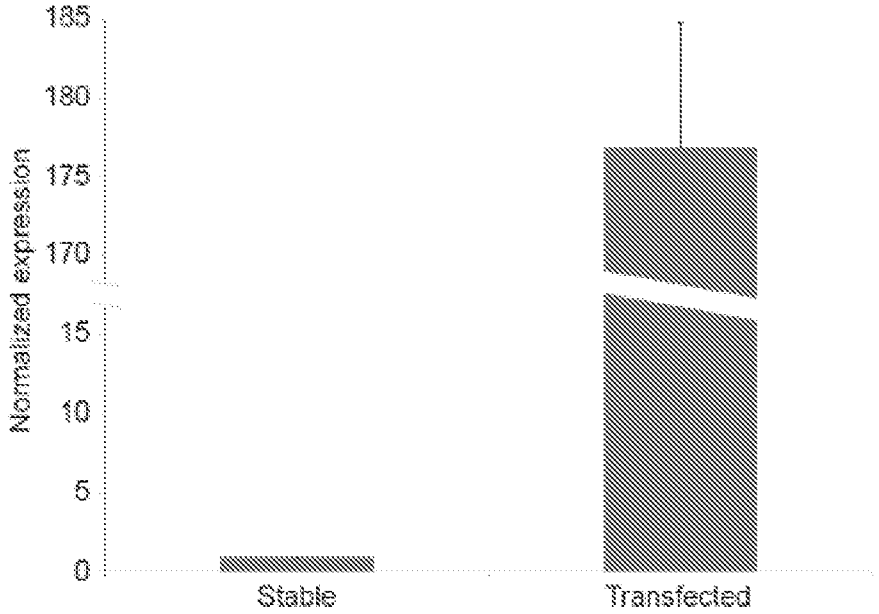

185
180
175
170
15
10
5
0
Normalized expression
Stable
Transfected

FIG. 29

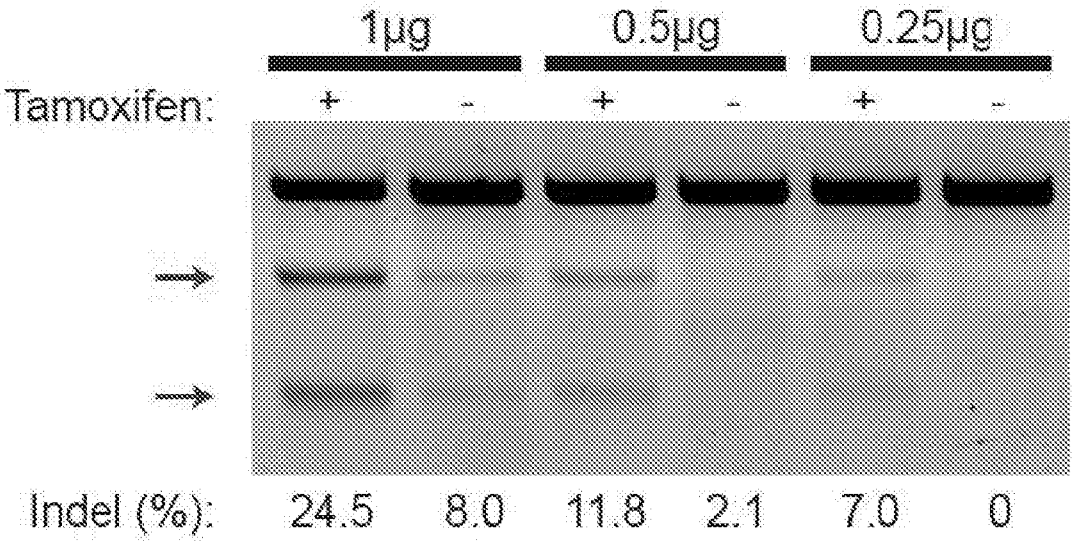

1µg
0.5µg
0.25µg
Tamoxifen:
+
-
+
-
+
-
Indel (%):
24.5
8.0
11.8
2.1
7.0
0

FIG. 30

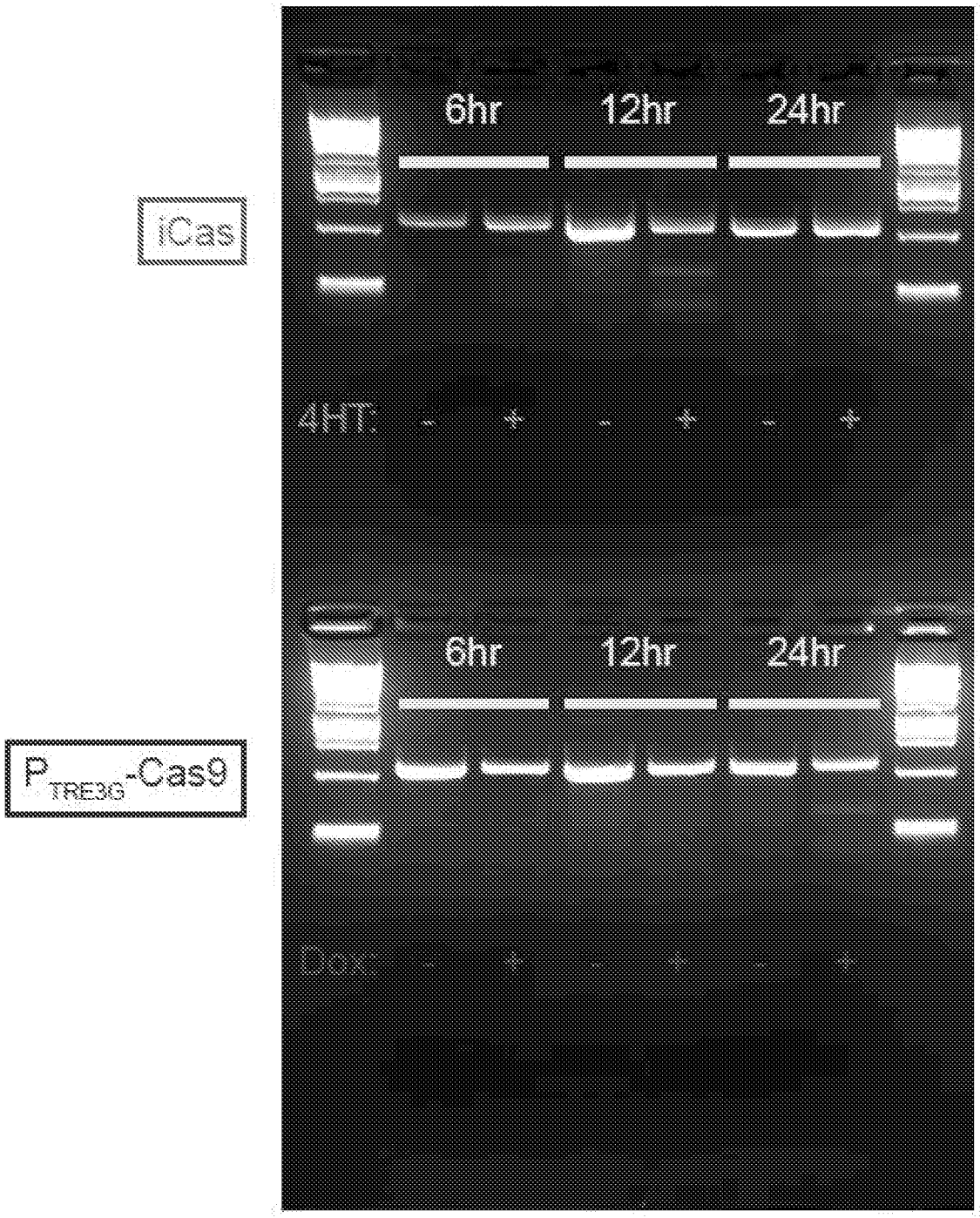


FIG. 31

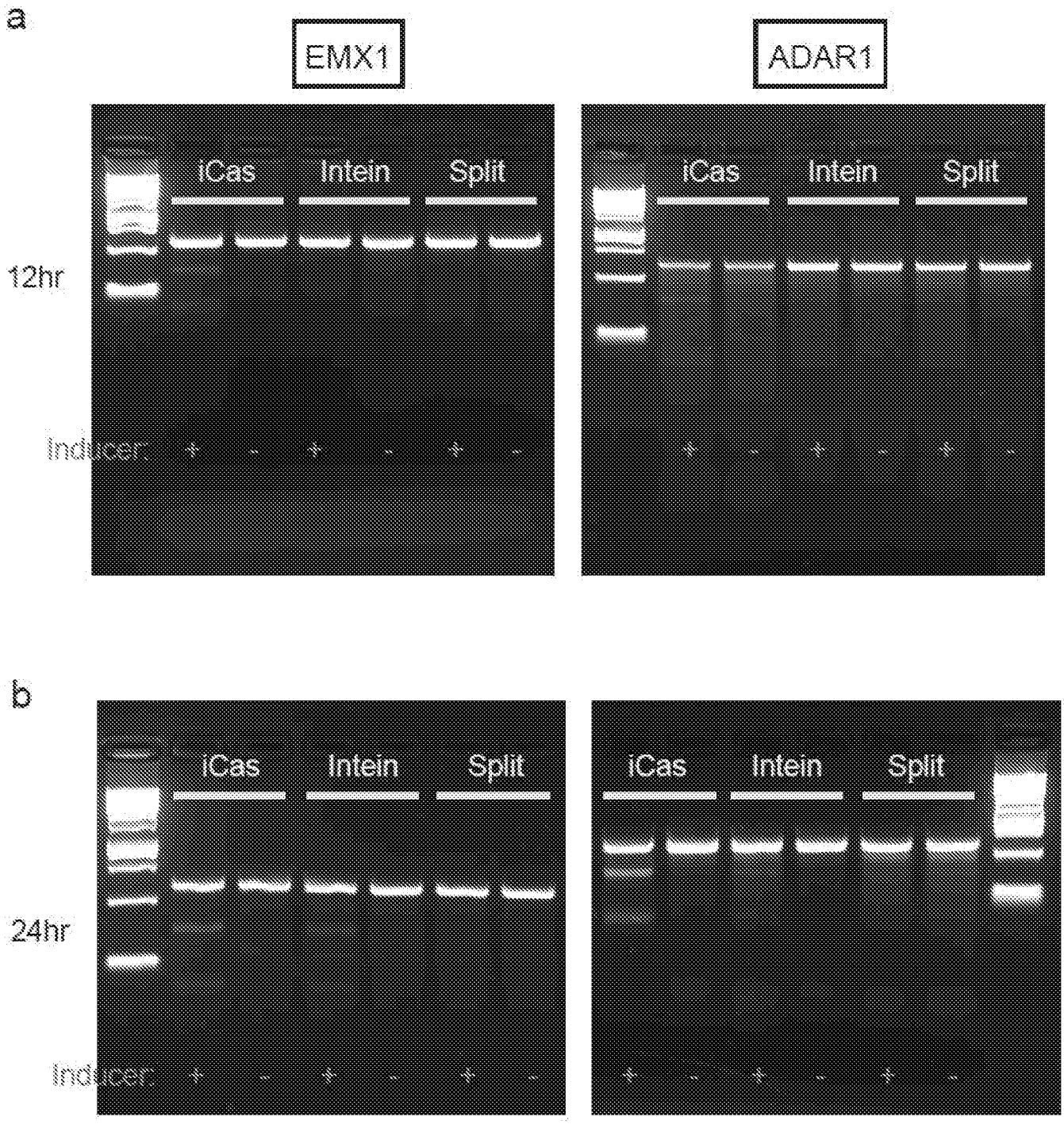


FIG. 32

