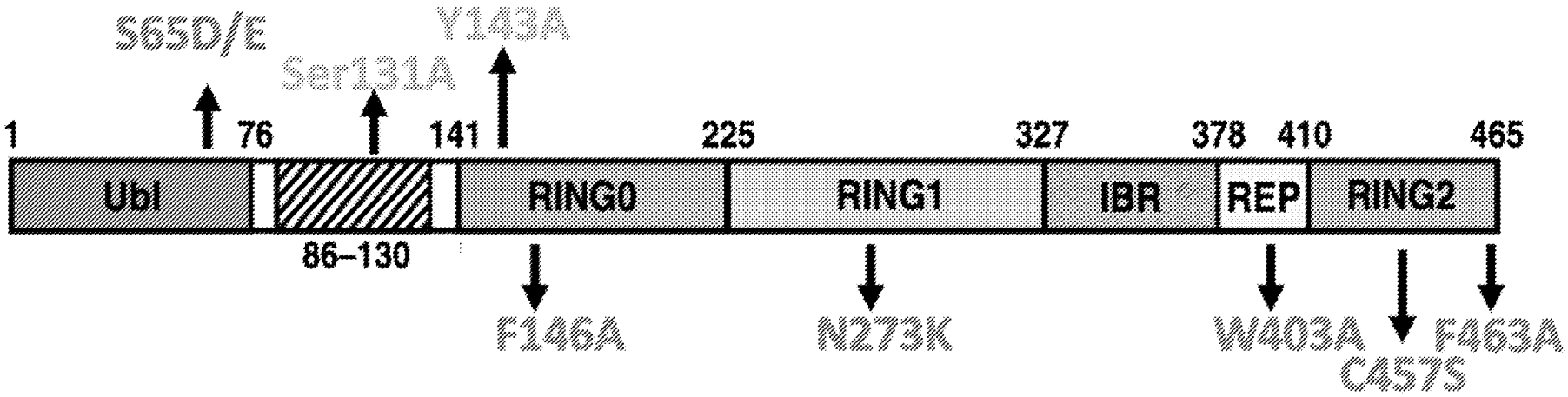

S65D/E
Y143A
Ser131A
1
76
141
225
327
378
410
465
Ubl
RING0
RING1
IBR
REP
RING2
86–130
F146A
N273K
W403A
F463A
C457S

FIG. 1


ITR
Enh
Promoter
5' UTR
Parkin
3' UTR
pA
ITR

FIG. 2

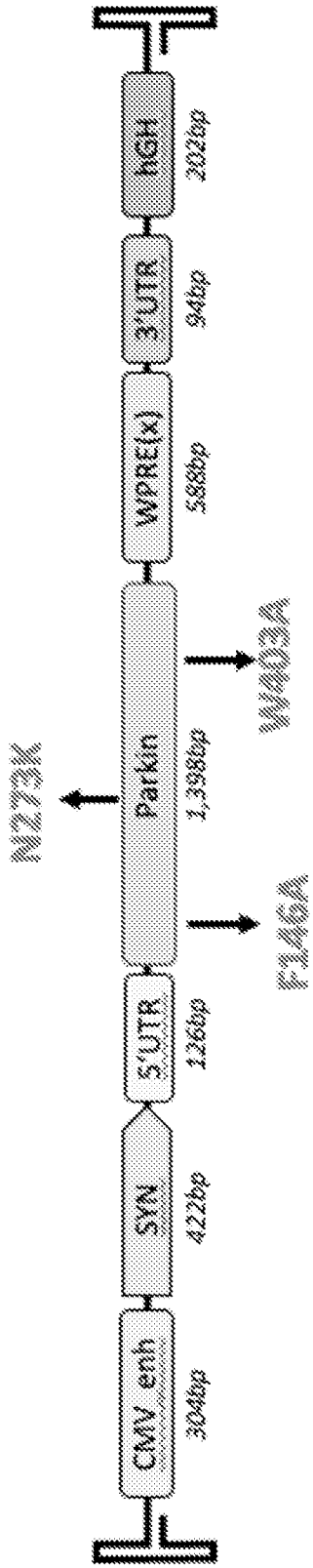

CMV_enh
304bp
SYN
422bp
5'UTR
126bp
Parkin
1,398bp
WPRE(x)
588bp
3'UTR
94bp
hGH
202bp
N273K
F146A
W403A

FIG. 3

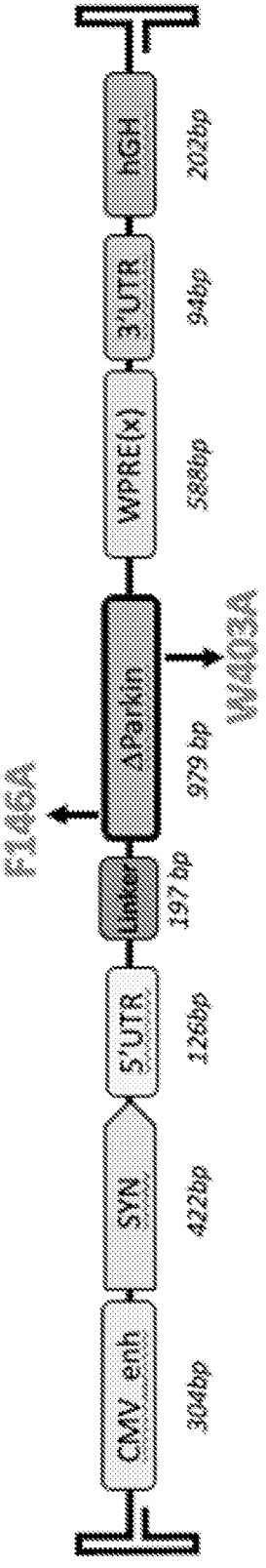

CMV_enh
304bp
SYN
422bp
5'UTR
126bp
Linker
197 bp
ΔParkin
979 bp
WPRE(x)
588bp
3'UTR
94bp
hGH
202bp
F146A
W403A

FIG. 4

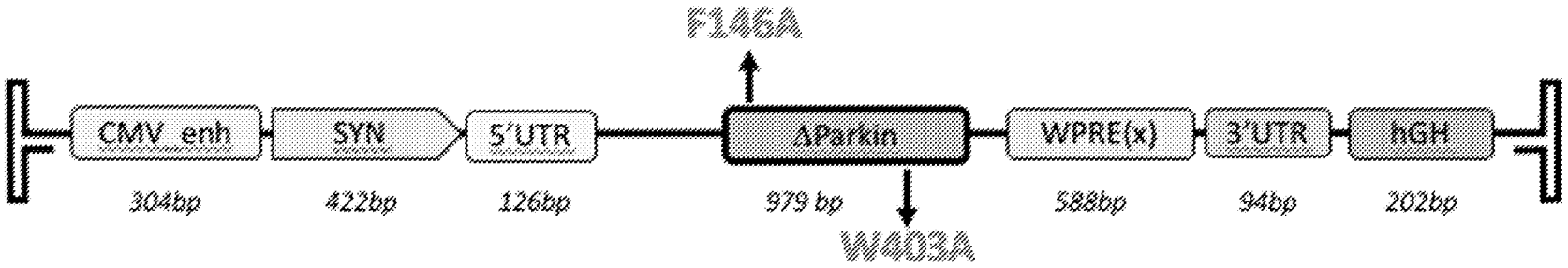

F146A
CMV_enh
SYN
5'UTR
ΔParkin
WPRE(x)
3'UTR
hGH
304bp
422bp
126bp
979 bp
588bp
94bp
202bp
W403A

FIG. 5



FIG. 6

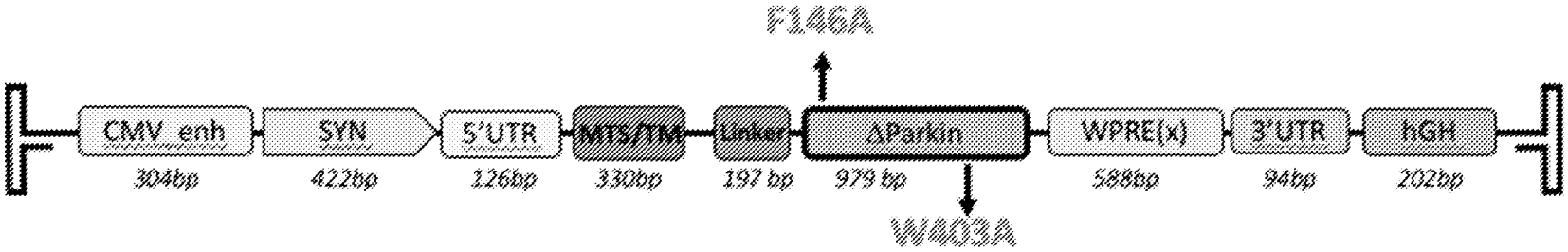


FIG. 7

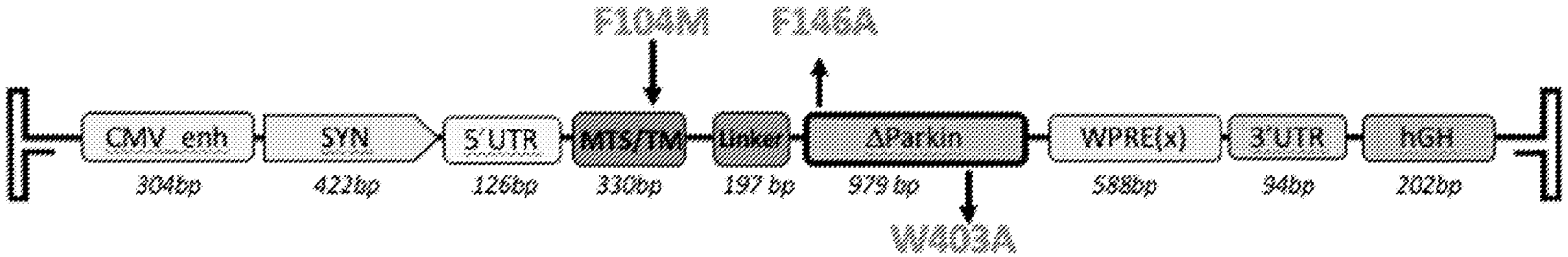


FIG. 8

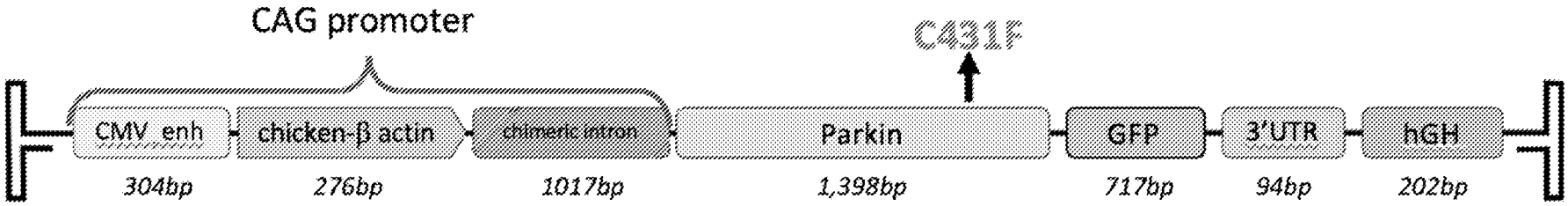

CAG promoter
C431F
CMV_enh
chicken-β actin
chimeric intron
Parkin
GFP
3'UTR
hGH
304bp
276bp
1017bp
1,398bp
717bp
94bp
202bp

FIG. 9

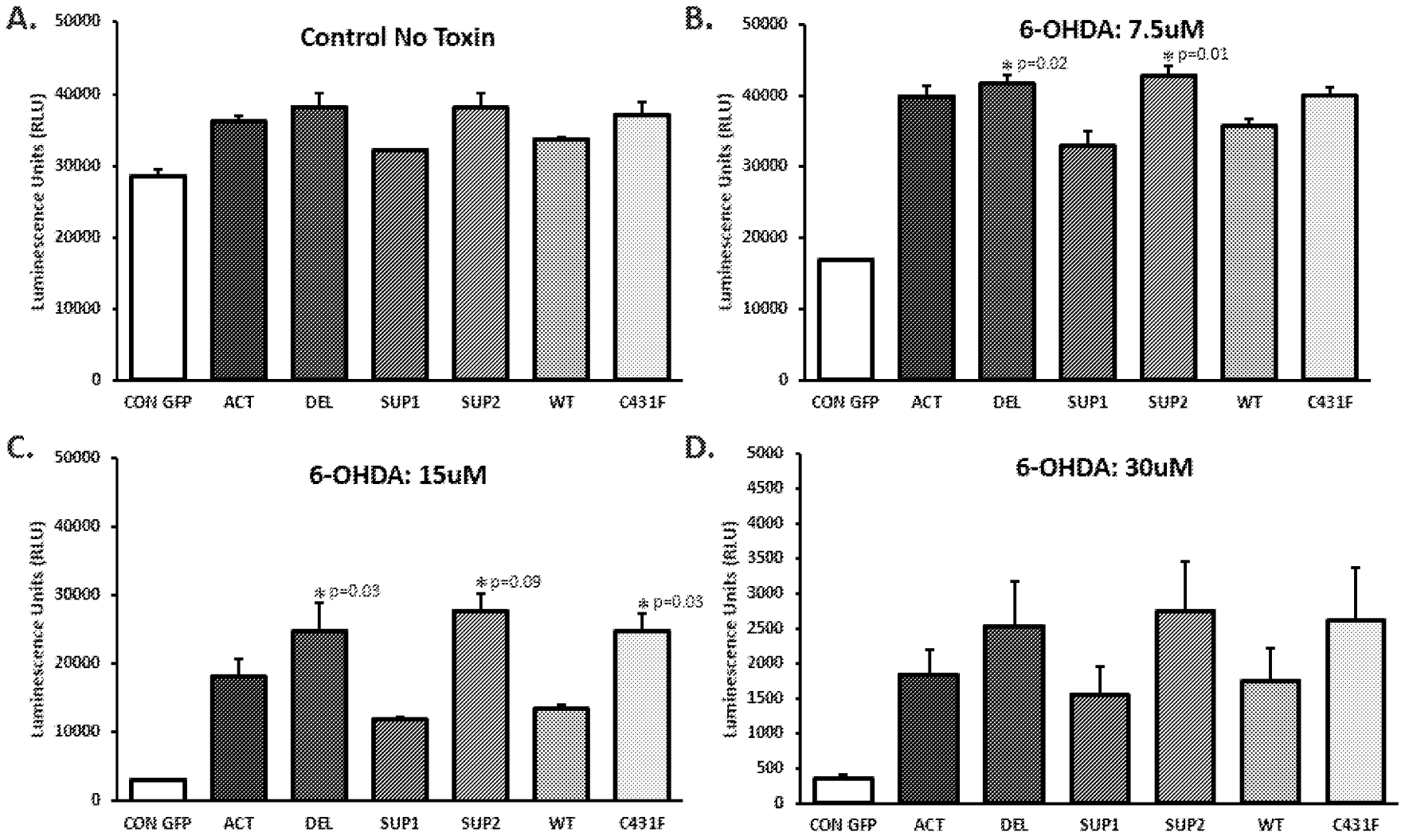

A.
Control No Toxin
Luminescence Units (RLU)
50000
40000
30000
20000
10000
0
CON GFP
ACT
DEL
SUP1
SUP2
WT
C431F
B.
6-OHDA: 7.5uM
* p=0.02
* p=0.01
C.
6-OHDA: 15uM
* p=0.03
* p=0.09
* p=0.03
D.
6-OHDA: 30uM
5000
4500
4000
3500
3000
2500
2000
1500
1000
500

FIG. 10A-10D

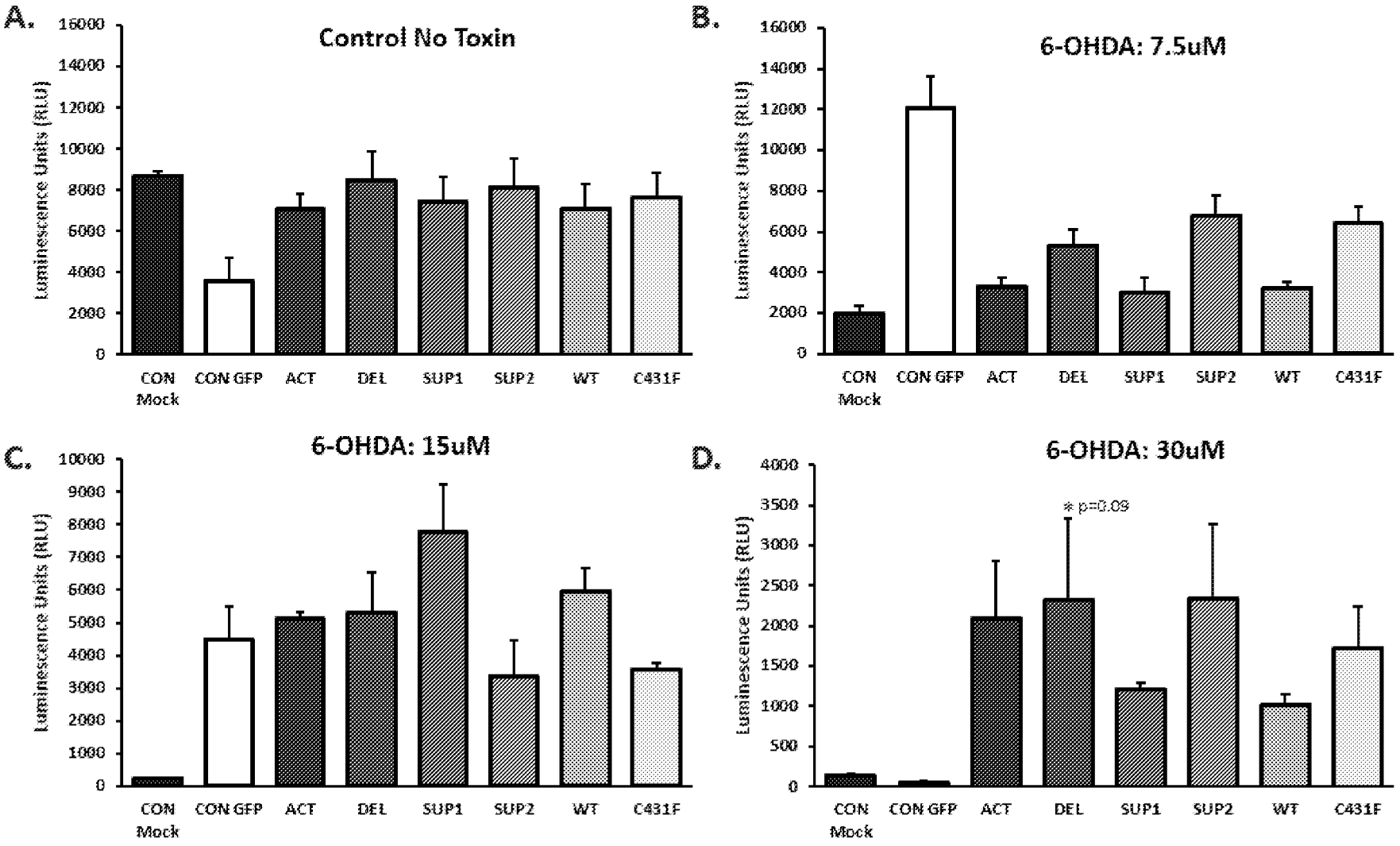

A.
Control No Toxin
Luminescence Units (RLU)
16000
14000
12000
10000
8000
6000
4000
2000
0
CON Mock
CON GFP
ACT
DEL
SUP1
SUP2
WT
C431F
B.
6-OHDA: 7.5uM
C.
6-OHDA: 15uM
9000
7000
5000
3000
1000
D.
6-OHDA: 30uM
4000
3500
3000
2500
2000
1500
1000
500
* p=0.09

FIG. 11A-11D

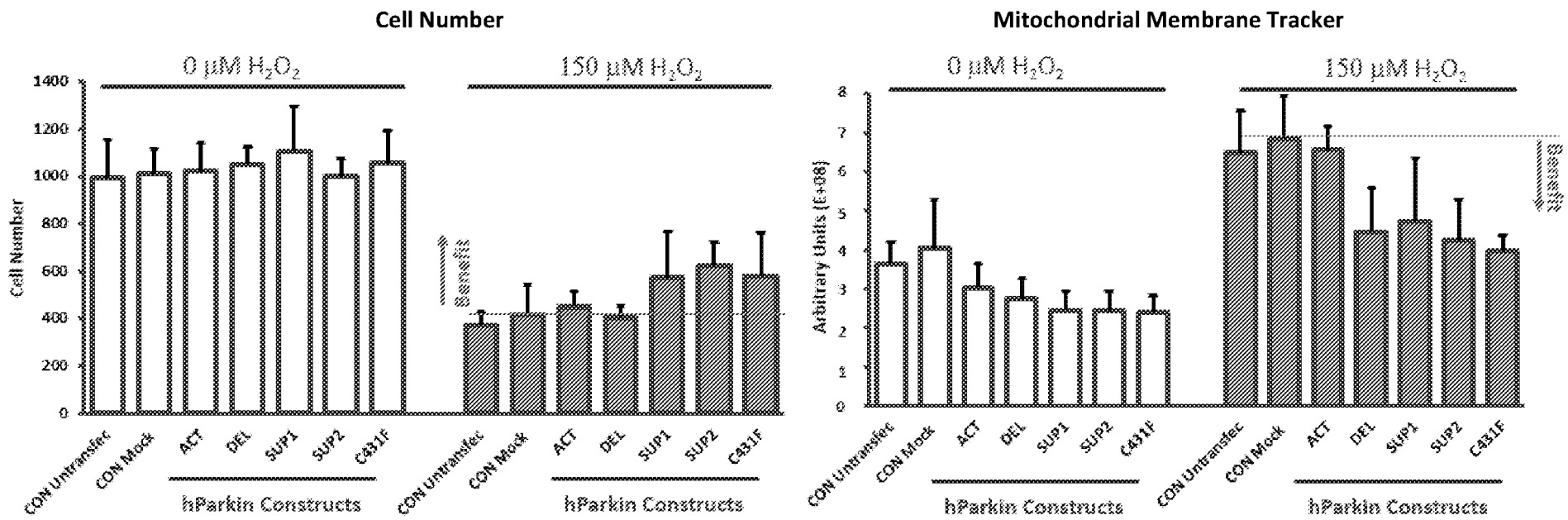
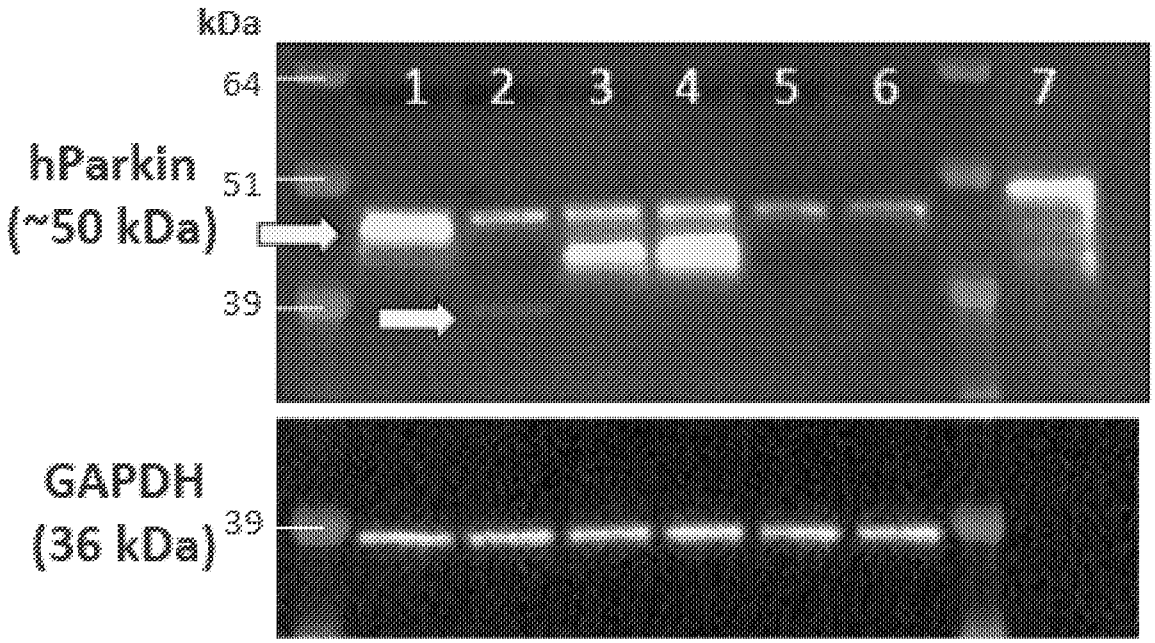

Cell Number
0 μM H_2O_2
150 μM H_2O_2
Cell Number
1400
1200
1000
800
600
400
200
0
Benefit
CON Untransfec
CON Mock
ACT
DEL
SUP1
SUP2
C431F
hParkin Constructs
Mitochondrial Membrane Tracker
0 μM H_2O_2
150 μM H_2O_2
Arbitrary Units (E+08)
8
7
6
5
4
3
2
1
0
Benefit
CON Untransfec
CON Mock
ACT
DEL
SUP1
SUP2
C431F
hParkin Constructs

FIG. 12



10µg Total Protein/Lane **Expected MW (kDa)**

Lane 1 – ACT ---------- 52.47
Lane 2 – DEL ---------- 36.34
Lane 3 – SUP1---------- 54.3 full, 43.42 cleaved
Lane 4 – SUP2---------- 54.3 full, 43.42 cleaved
Lane 5 – CON GFP
Lane 6 – CON Non-transduced
Ladder
Lane 7 –recomb.hPARKIN-Flag (10pg) ---- 55.25

FIG. 13

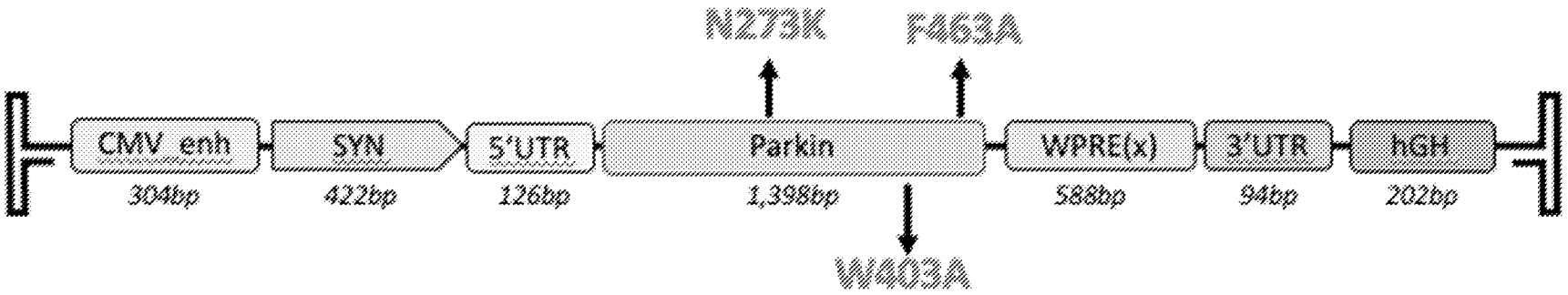

N273K
F463A
CMV_enh
SYN
5'UTR
Parkin
WPRE(x)
3'UTR
hGH
304bp
422bp
126bp
1,398bp
588bp
94bp
202bp
W403A

FIG. 14

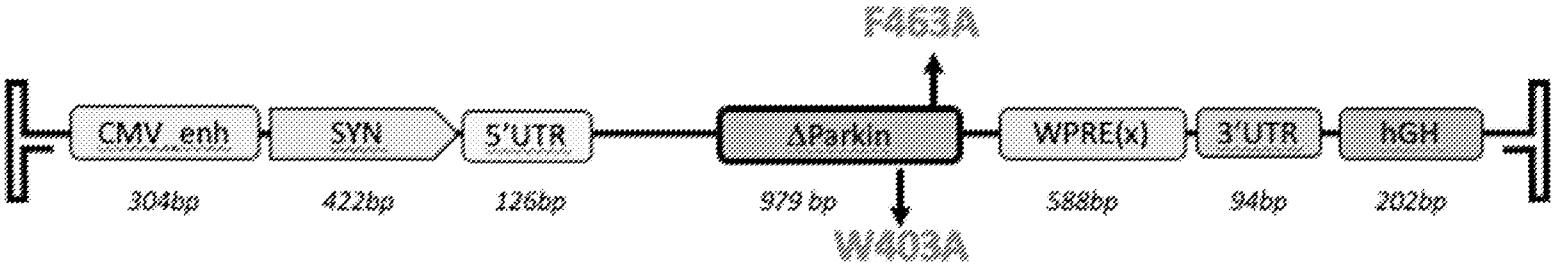

F463A
CMV_enh
SYN
5'UTR
ΔParkin
WPRE(x)
3'UTR
hGH
304bp
422bp
126bp
979 bp
588bp
94bp
202bp
W403A

FIG. 15

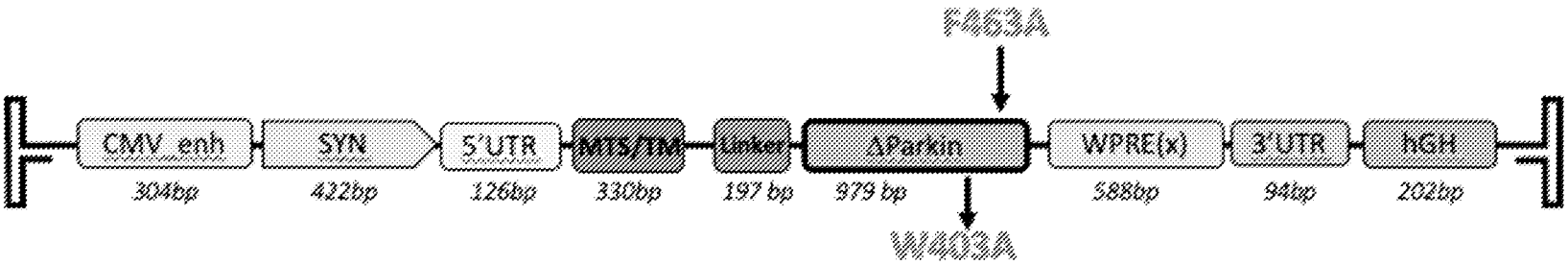

F463A
CMV_enh
304bp
SYN
422bp
5'UTR
126bp
MTS/TM
330bp
Linker
197 bp
ΔParkin
979 bp
W403A
WPRE(x)
588bp
3'UTR
94bp
hGH
202bp

FIG. 16

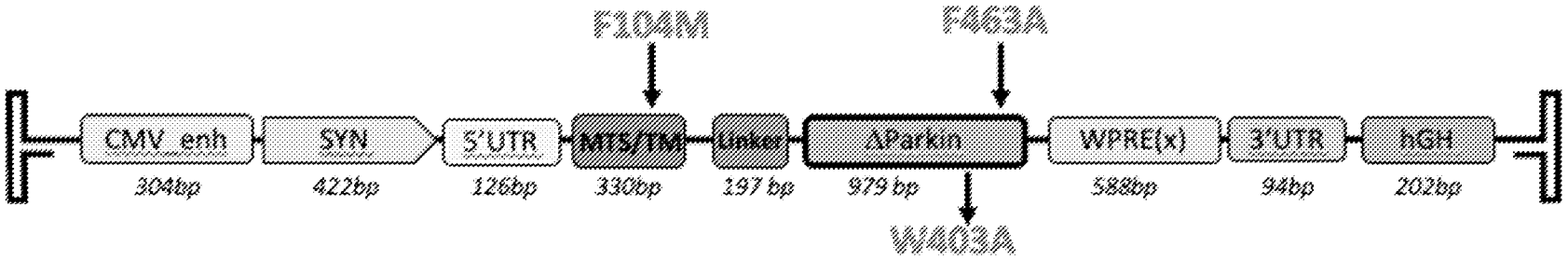

F104M
F463A
CMV_enh
304bp
SYN
422bp
5'UTR
126bp
MTS/TM
330bp
Linker
197 bp
ΔParkin
979 bp
W403A
WPRE(x)
588bp
3'UTR
94bp
hGH
202bp

FIG. 17