FIGURE 1

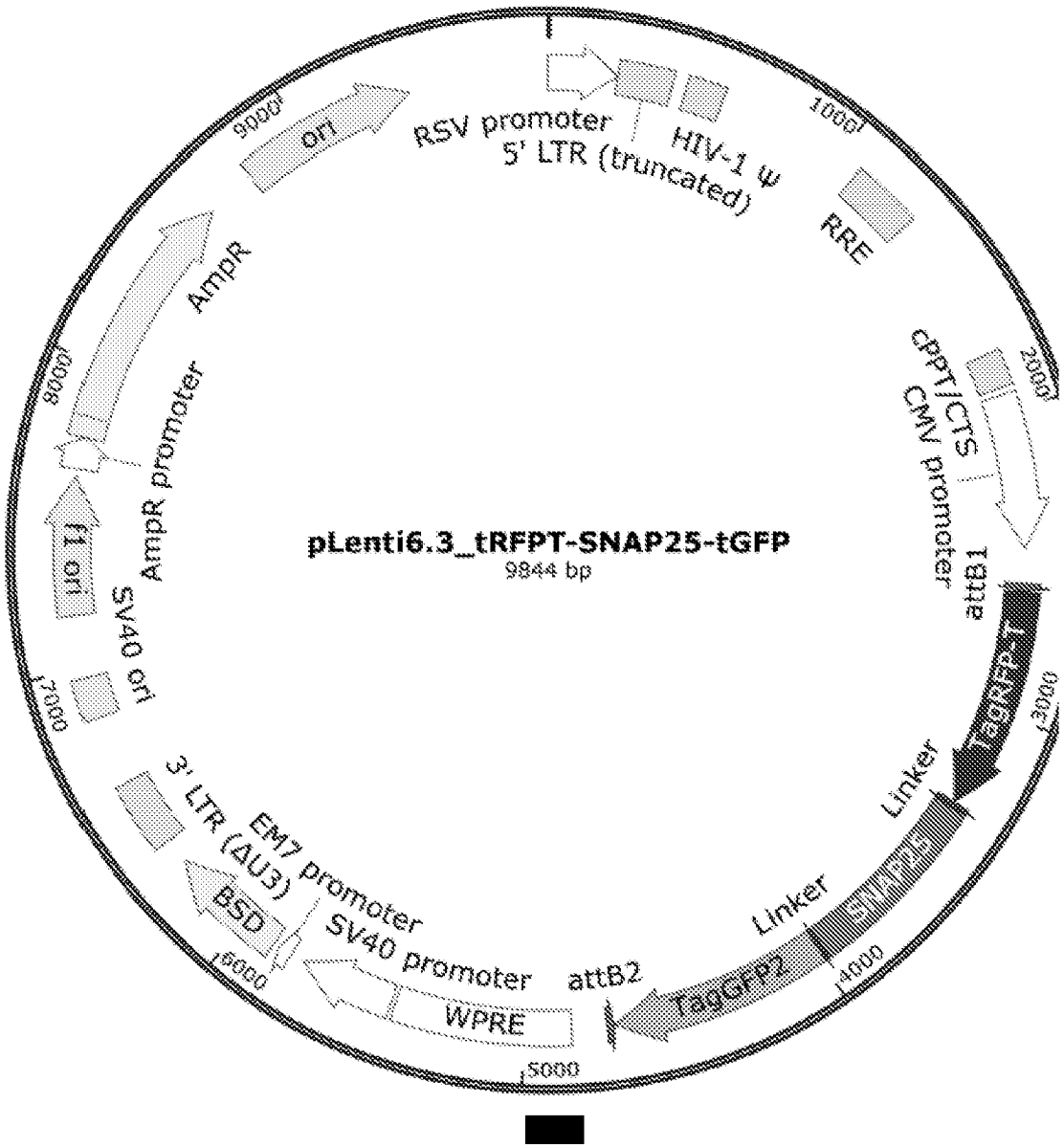

pLenti6.3_tRFPT-SNAP25-tGFP
9844 bp
1000
2000
3000
4000
5000
6000
7000
8000
9000
ori
RSV promoter
5' LTR (truncated)
HIV-1 ψ
RRE
cPPT/CTS
CMV promoter
attB1
TagRFP-T
Linker
SNAP25
Linker
TagGFP2
attB2
WPRE
SV40 promoter
EM7 promoter
BSD
3' LTR (ΔU3)
SV40 ori
f1 ori
AmpR promoter
AmpR

FIGURE 1 CONTINUED

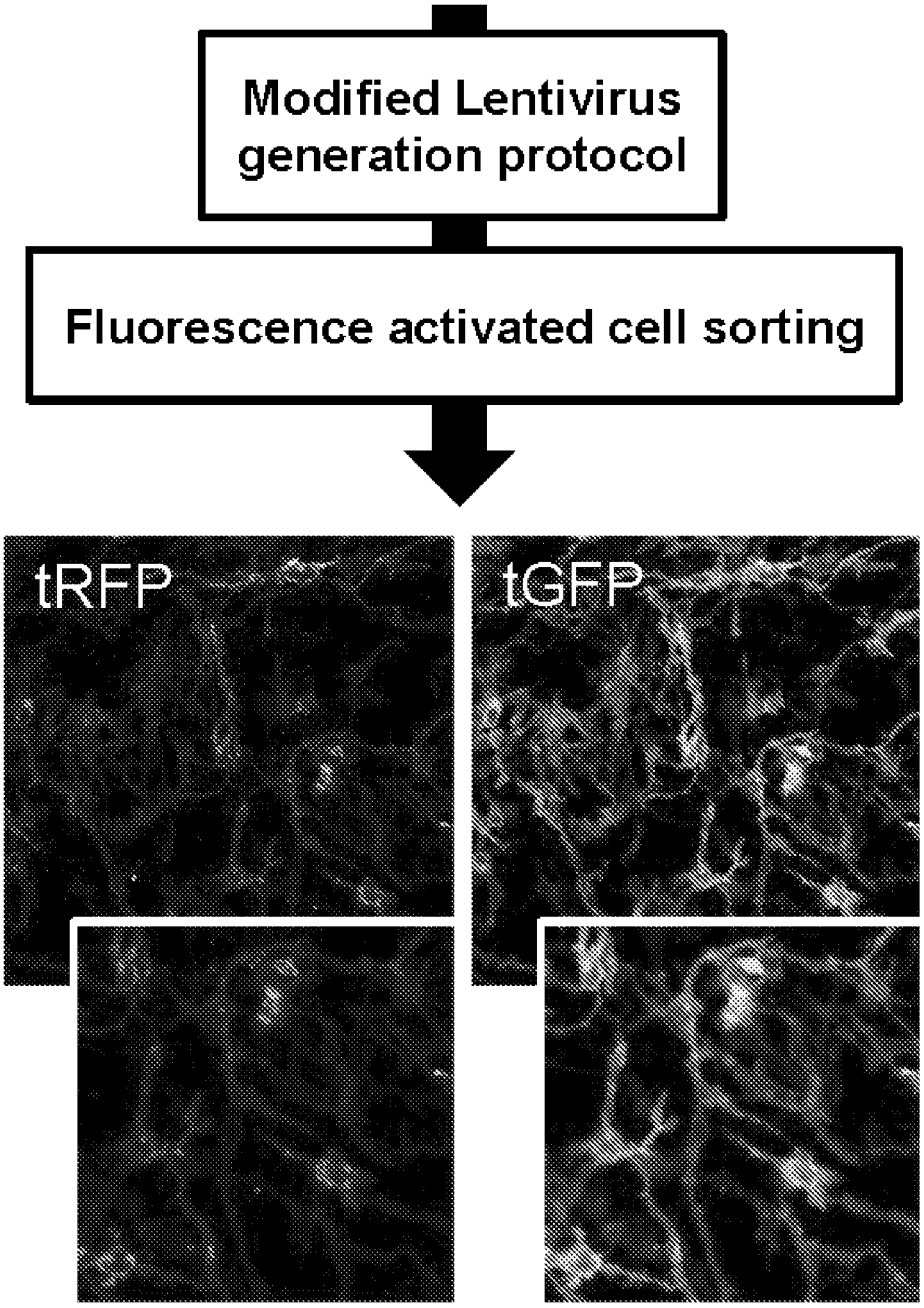


FIGURE 2

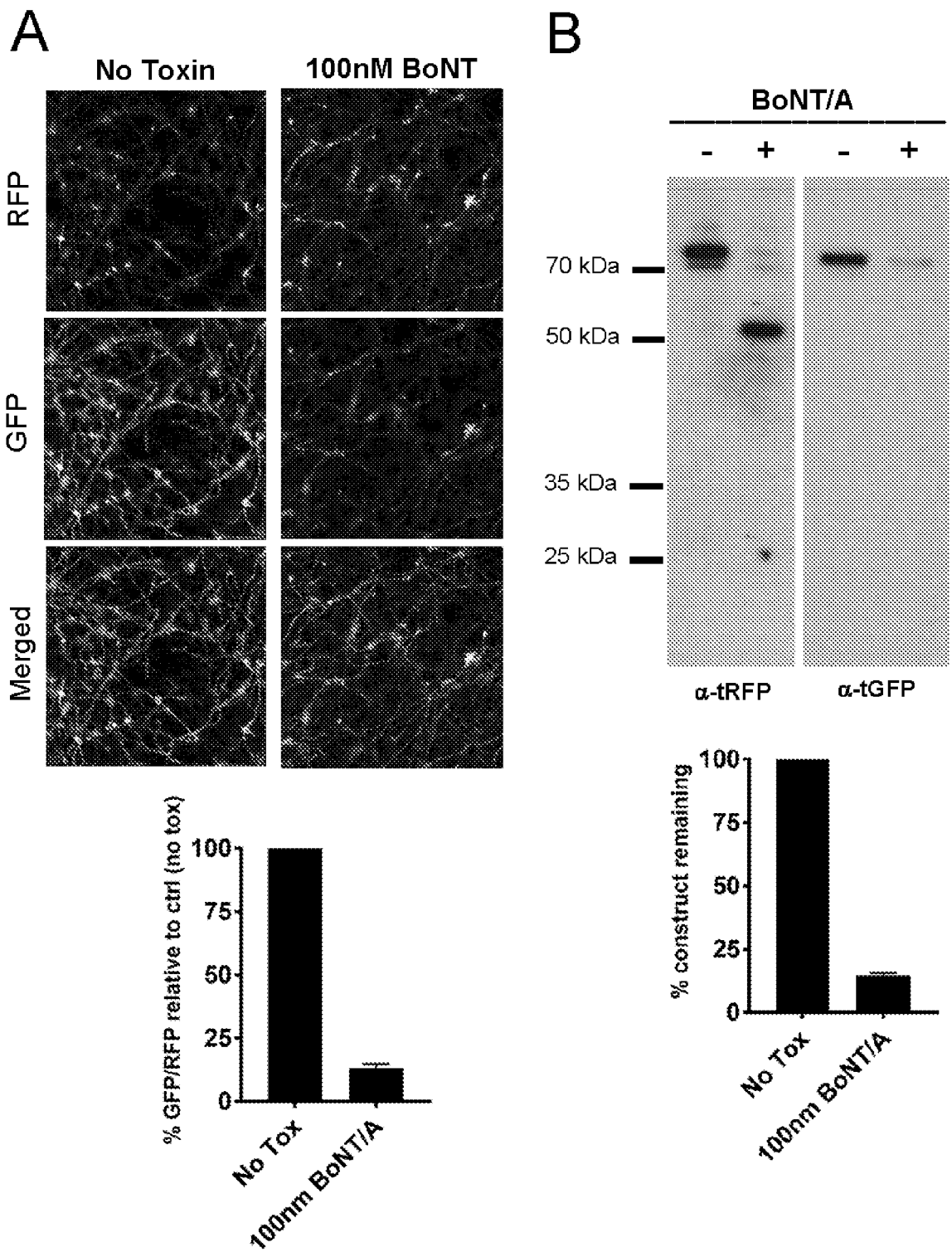

A
No Toxin
100nM BoNT
RFP
GFP
Merged
% GFP/RFP relative to ctrl (no tox)
100
75
50
25
0
No Tox
100nm BoNT/A
B
BoNT/A
-
+
-
+
70 kDa
50 kDa
35 kDa
25 kDa
α-tRFP
α-tGFP
% construct remaining
100
75
50
25
0
No Tox
100nm BoNT/A

FIGURE 3

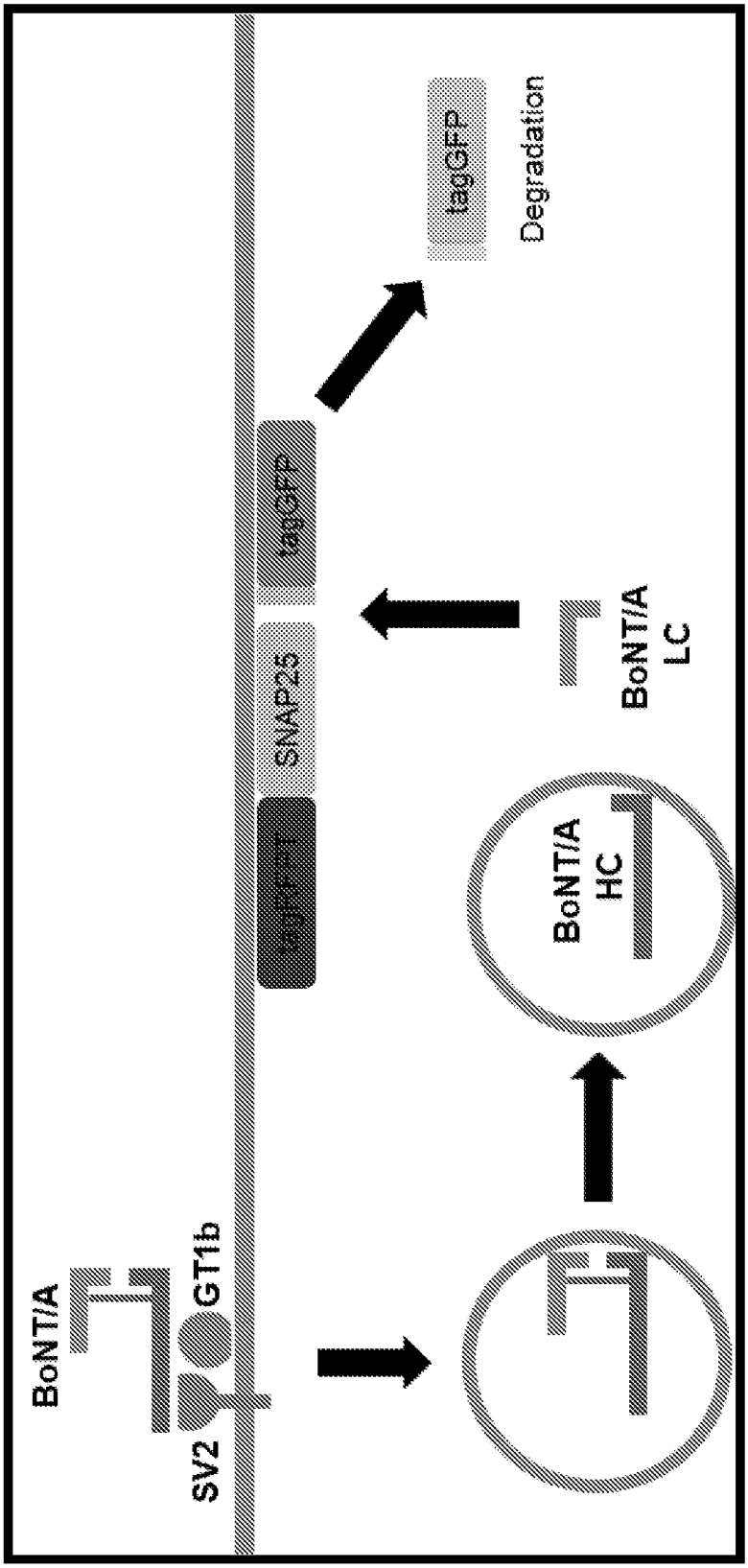

BoNT/A
GT1b
SV2
tagFRET
SNAP25
tagGFP
tagGFP
Degradation
BoNT/A
LC
BoNT/A
HC

FIGURE 4

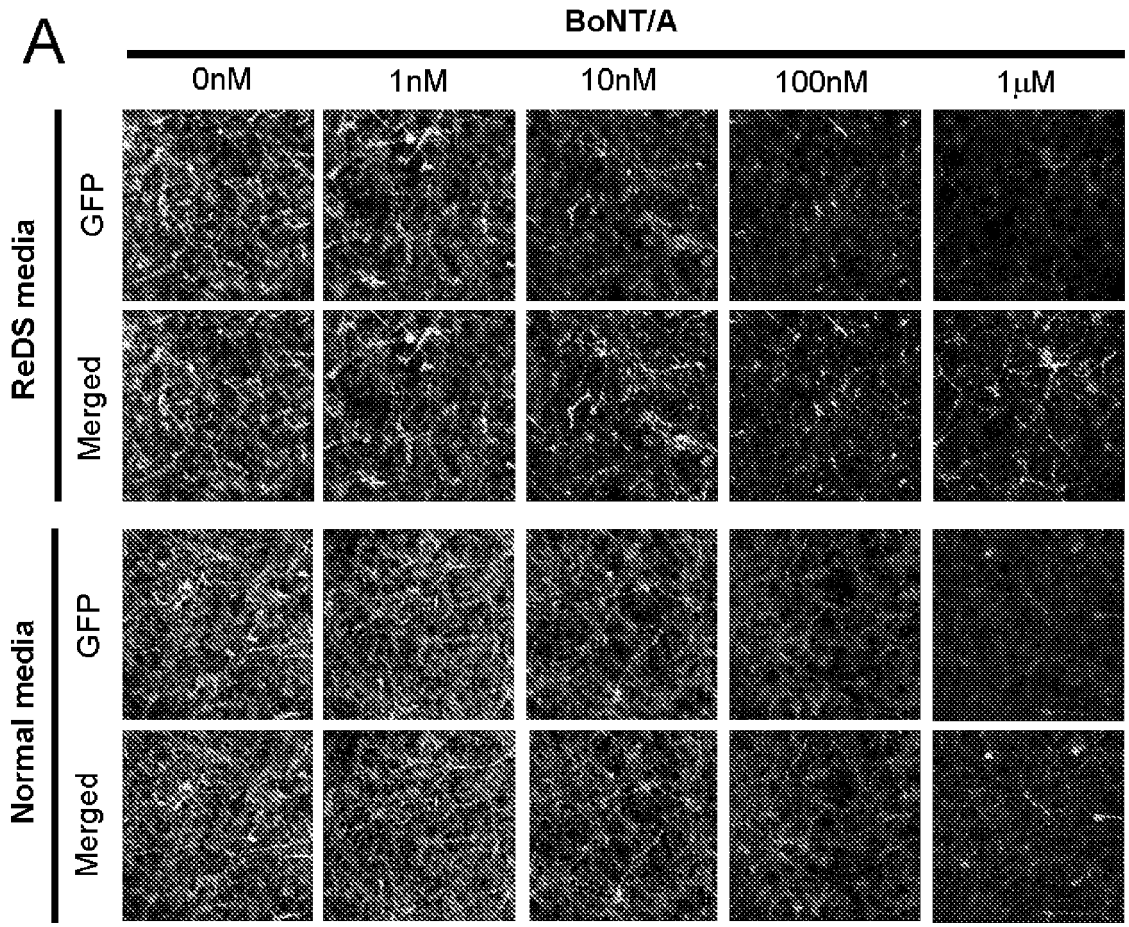

A
BoNT/A
0nM
1nM
10nM
100nM
1μM
ReDS media
GFP
Merged
Normal media
GFP
Merged

FIGURE 4 CONTINUED

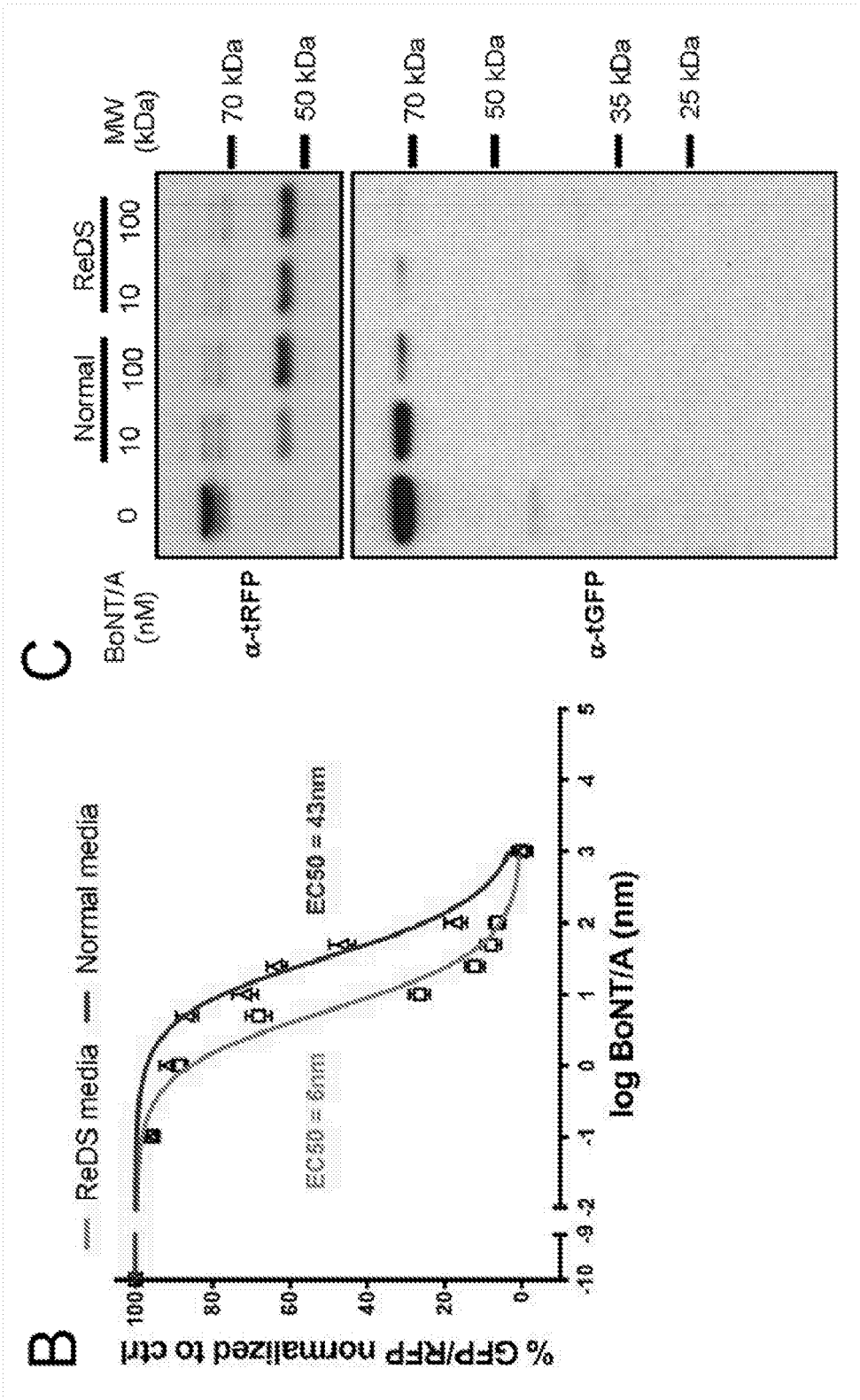


FIGURE 5

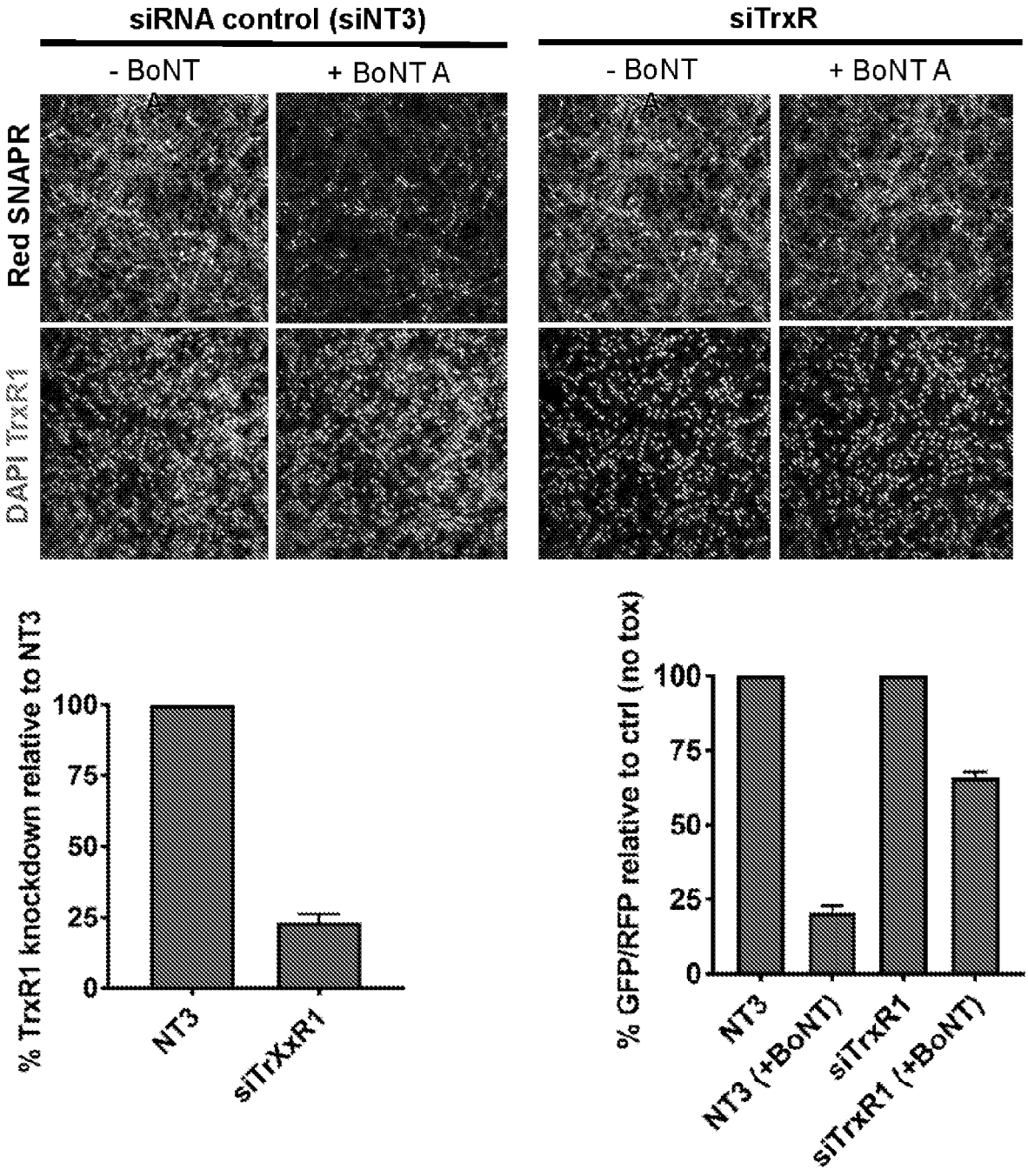

siRNA control (siNT3)
siTrxR
- BoNT
+ BoNT A
- BoNT
+ BoNT A
Red SNAPR
DAPI TrxR1
% TrxR1 knockdown relative to NT3
100
75
50
25
0
NT3
siTrXxR1
% GFP/RFP relative to ctrl (no tox)
100
75
50
25
0
NT3
NT3 (+BoNT)
siTrxR1
siTrxR1 (+BoNT)

FIGURE 6

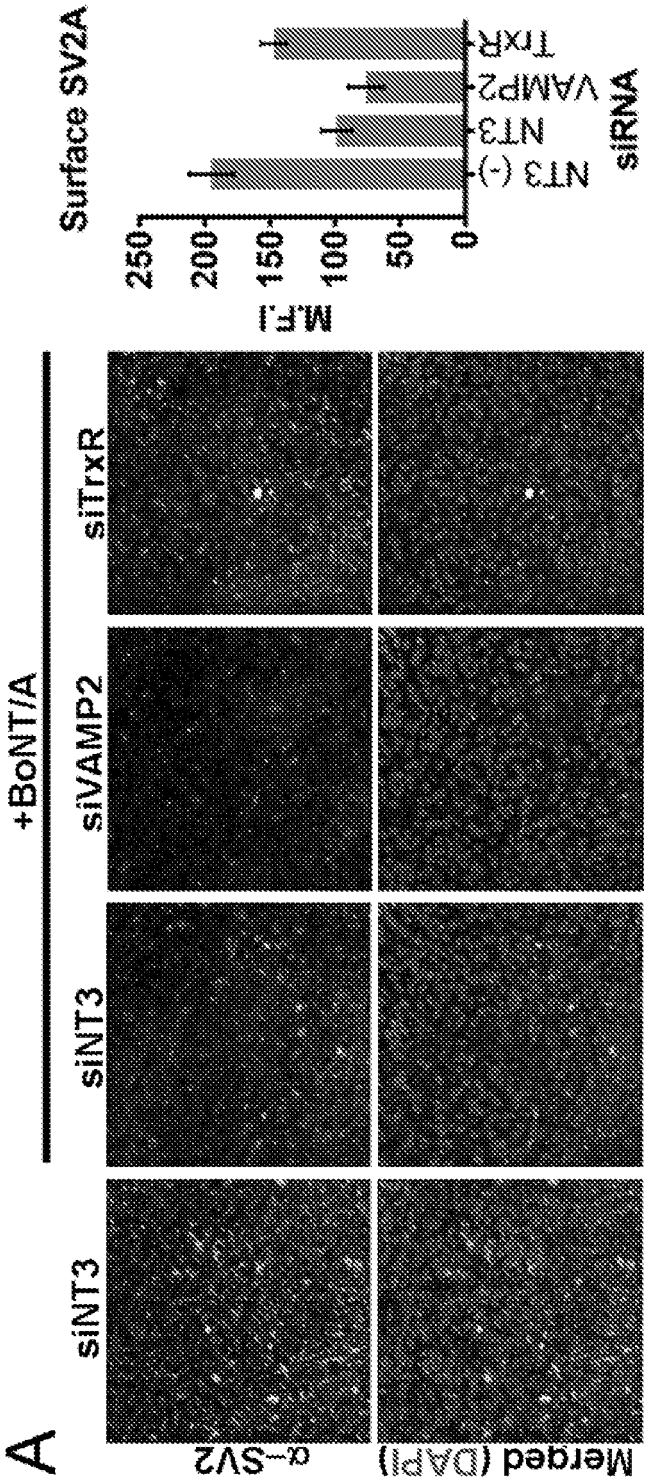

A
+BoNT/A
siNT3
siVAMP2
siTrxR
siNT3
α-SV2
Merged (DAPI)
Surface SV2A
M.F.I.
250
200
150
100
50
0
NT3 (-)
NT3
VAMP2
TrxR
siRNA

FIGURE 6 CONTINUED

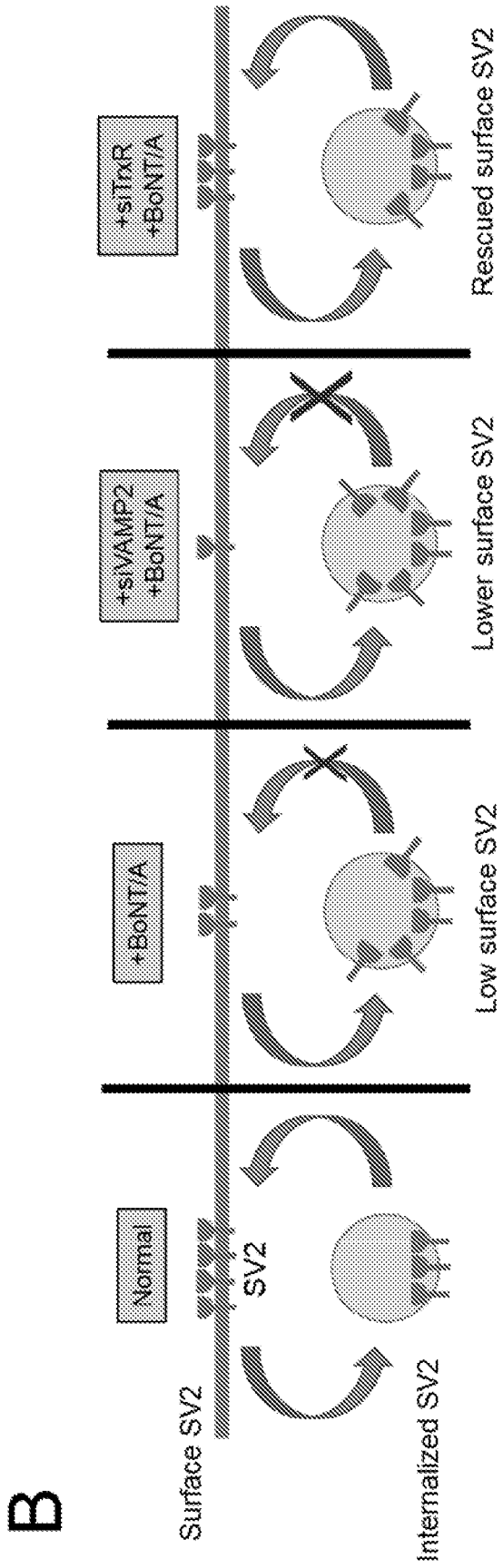


FIGURE 7

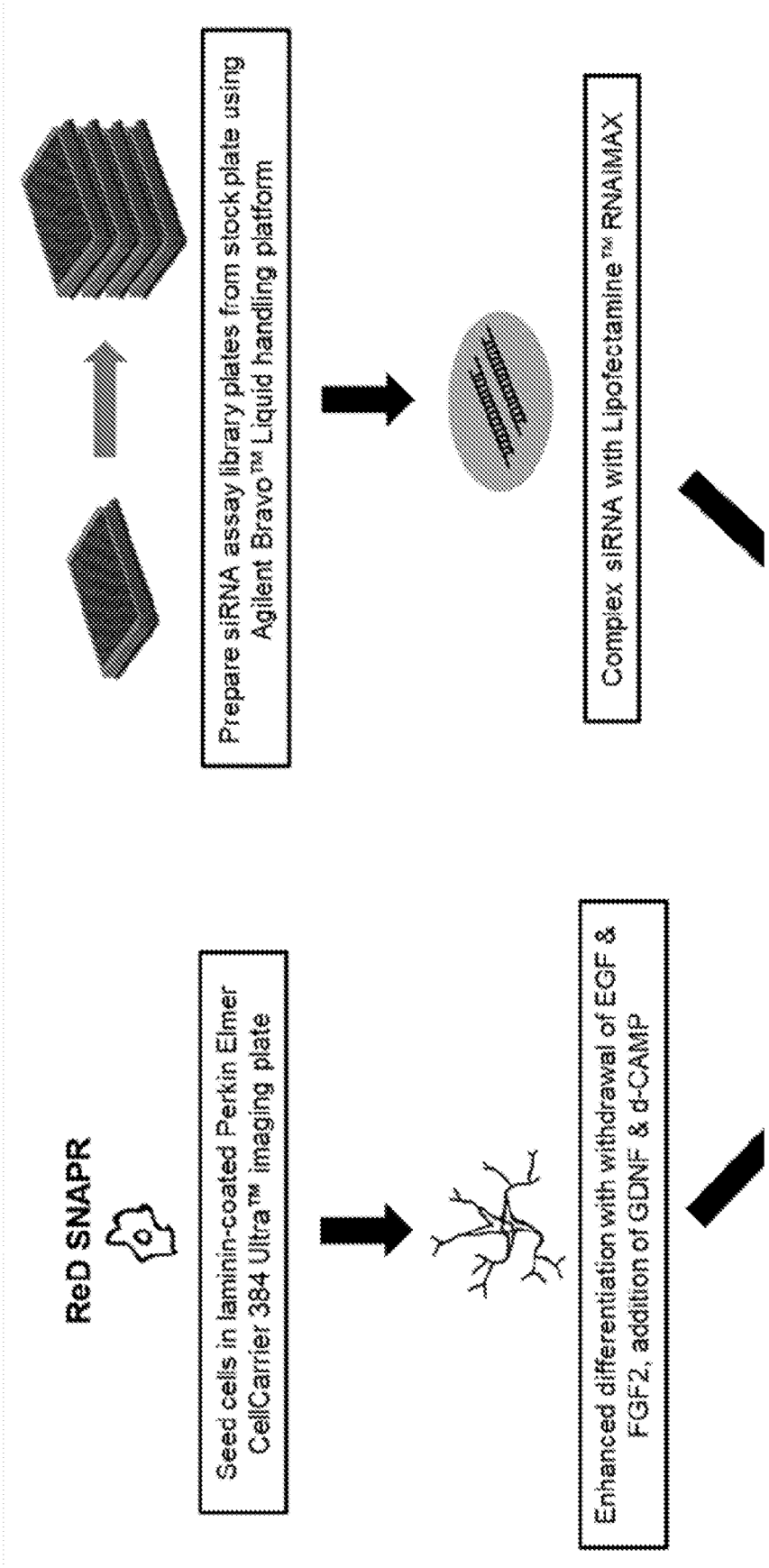

Prepare siRNA assay library plates from stock plate using Agilent Bravo™ Liquid handling platform
Complex siRNA with Lipofectamine™ RNAiMAX
ReD SNAPR
Seed cells in laminin-coated Perkin Elmer CellCarrier 384 Ultra™ imaging plate
Enhanced differentiation with withdrawal of EGF & FGF2, addition of GDNF & d-CAMP

FIGURE 7 CONTINUED

