Figure 1.

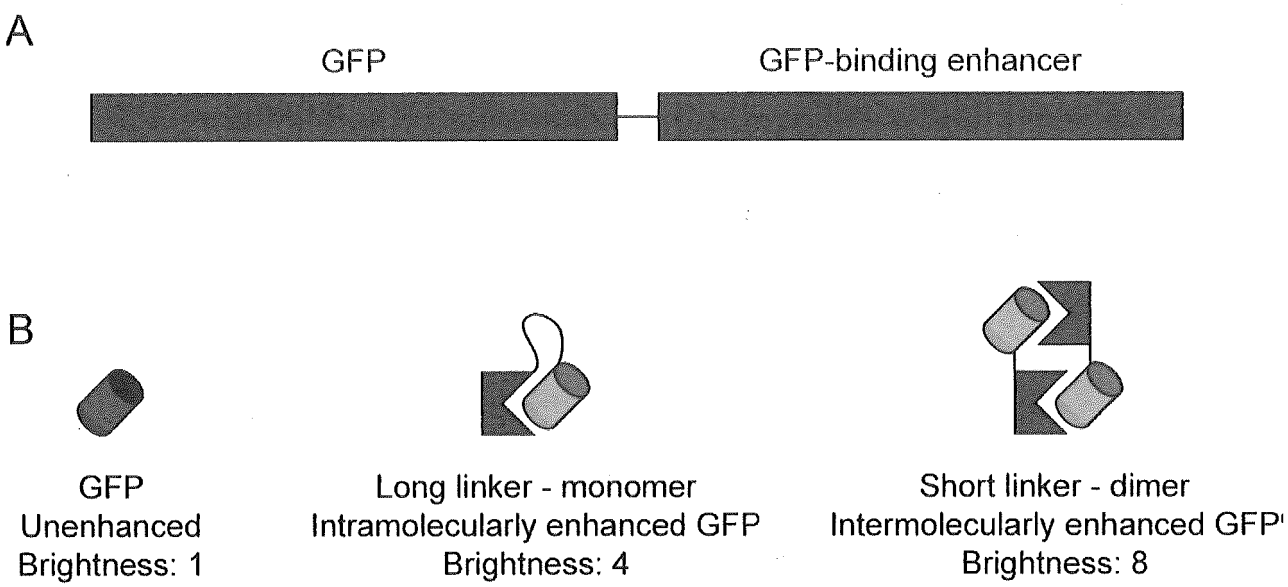

A
GFP
GFP-binding enhancer
B
GFP
Unenhanced
Brightness: 1
Long linker - monomer
Intramolecularly enhanced GFP
Brightness: 4
Short linker - dimer
Intermolecularly enhanced GFP
Brightness: 8

Figure 2

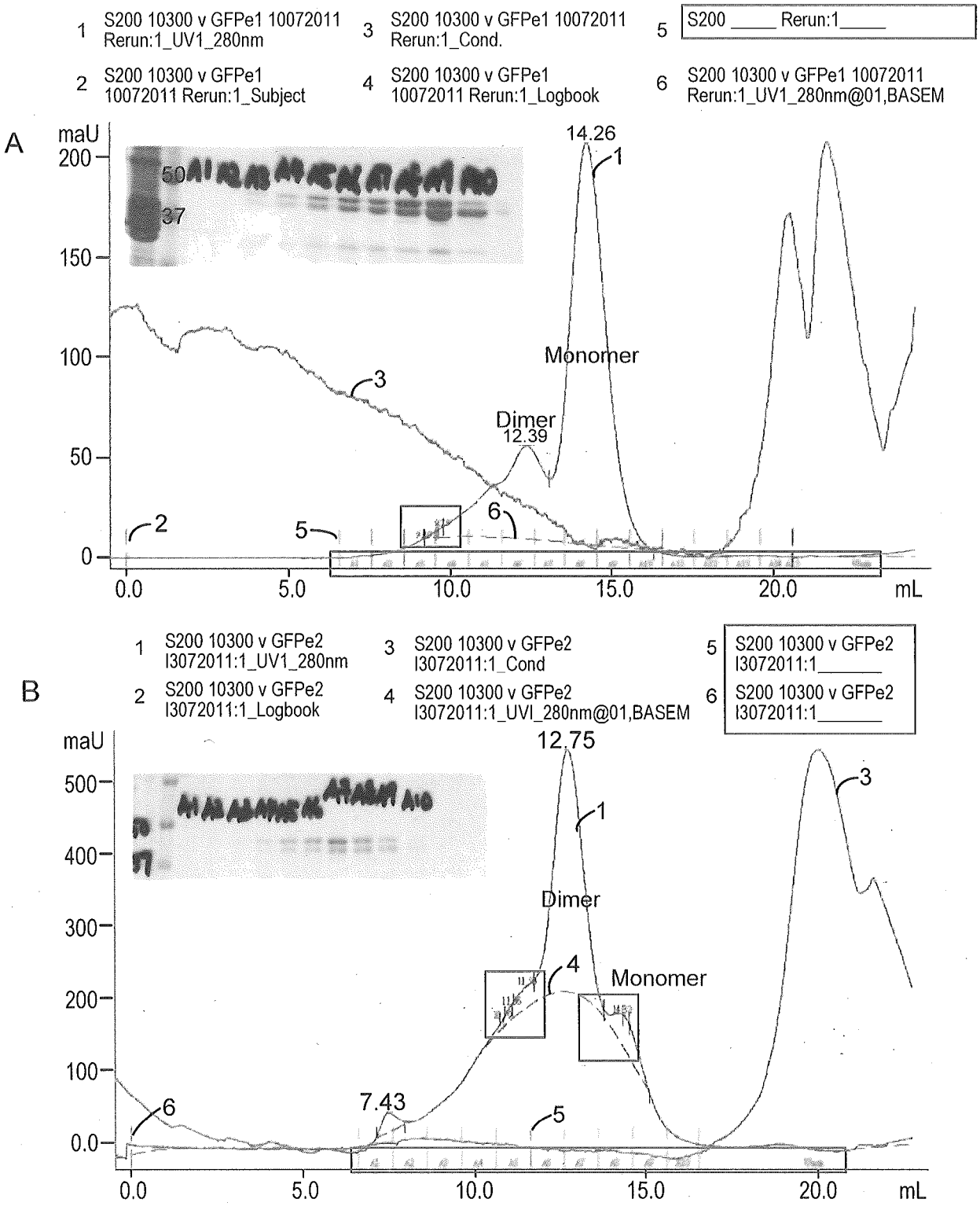

1 S200 10300 v GFPe1 10072011 Rerun:1_UV1_280nm
2 S200 10300 v GFPe1 10072011 Rerun:1_Subject
3 S200 10300 v GFPe1 10072011 Rerun:1_Cond.
4 S200 10300 v GFPe1 10072011 Rerun:1_Logbook
5 S200 _____ Rerun:1_____
6 S200 10300 v GFPe1 10072011 Rerun:1_UV1_280nm@01,BASEM
A
maU
200
150
100
50
0
14.26
1
Monomer
3
Dimer
12.39
2
5
6
0.0
5.0
10.0
15.0
20.0
mL
B
1 S200 10300 v GFPe2 I3072011:1_UV1_280nm
2 S200 10300 v GFPe2 I3072011:1_Logbook
3 S200 10300 v GFPe2 I3072011:1_Cond
4 S200 10300 v GFPe2 I3072011:1_UVI_280nm@01,BASEM
5 S200 10300 v GFPe2 I3072011:1_____
6 S200 10300 v GFPe2 I3072011:1_____
maU
500
400
300
200
100
0.0
12.75
1
3
Dimer
4
Monomer
7.43
6
5
0.0
5.0
10.0
15.0
20.0
mL

Figure 3

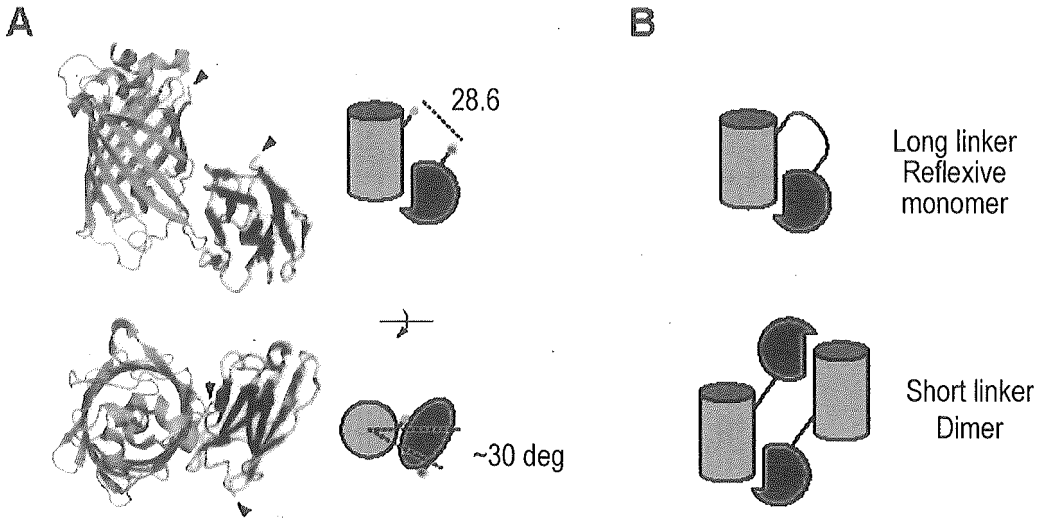


Figure 4

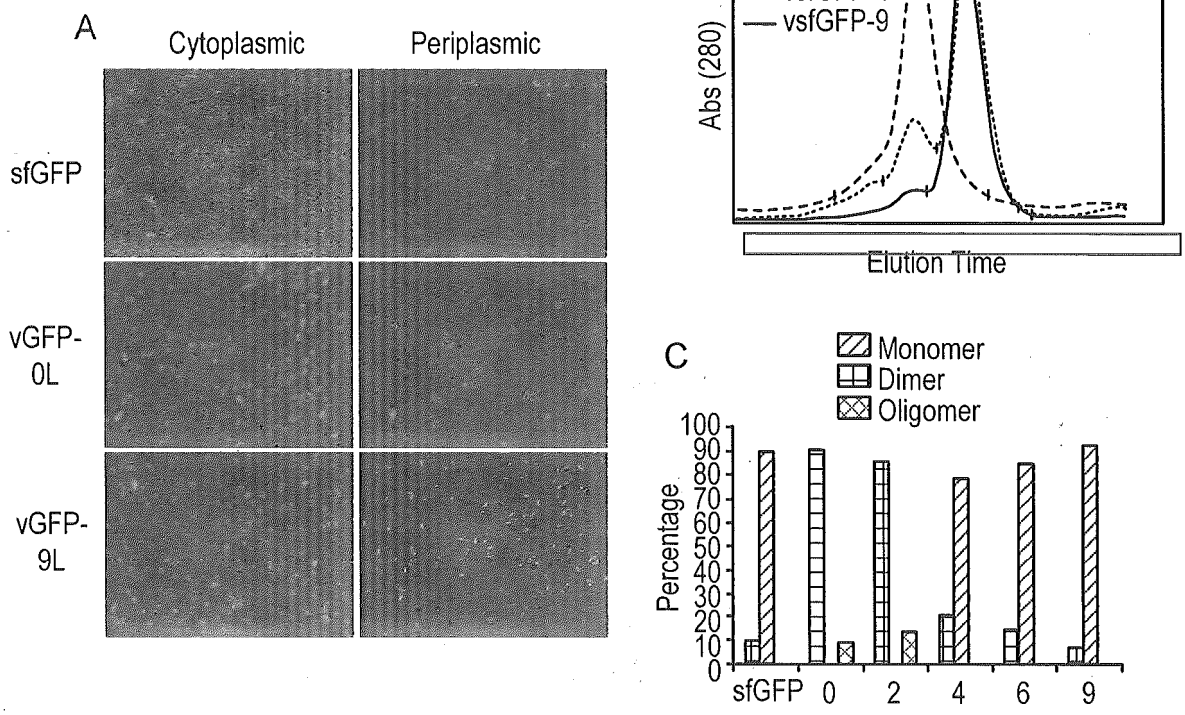


Figure 5

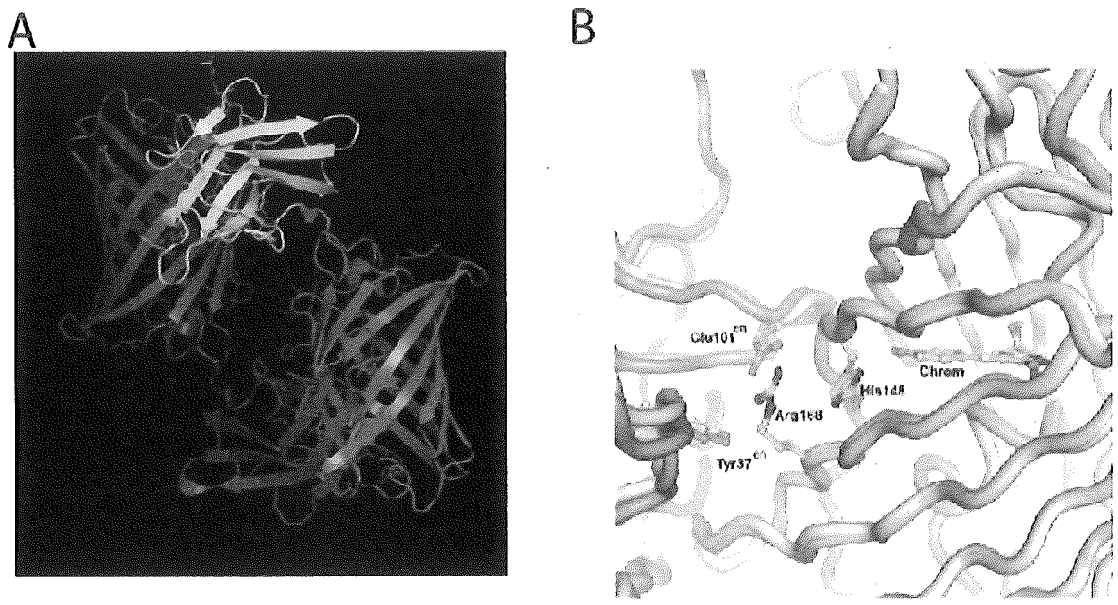
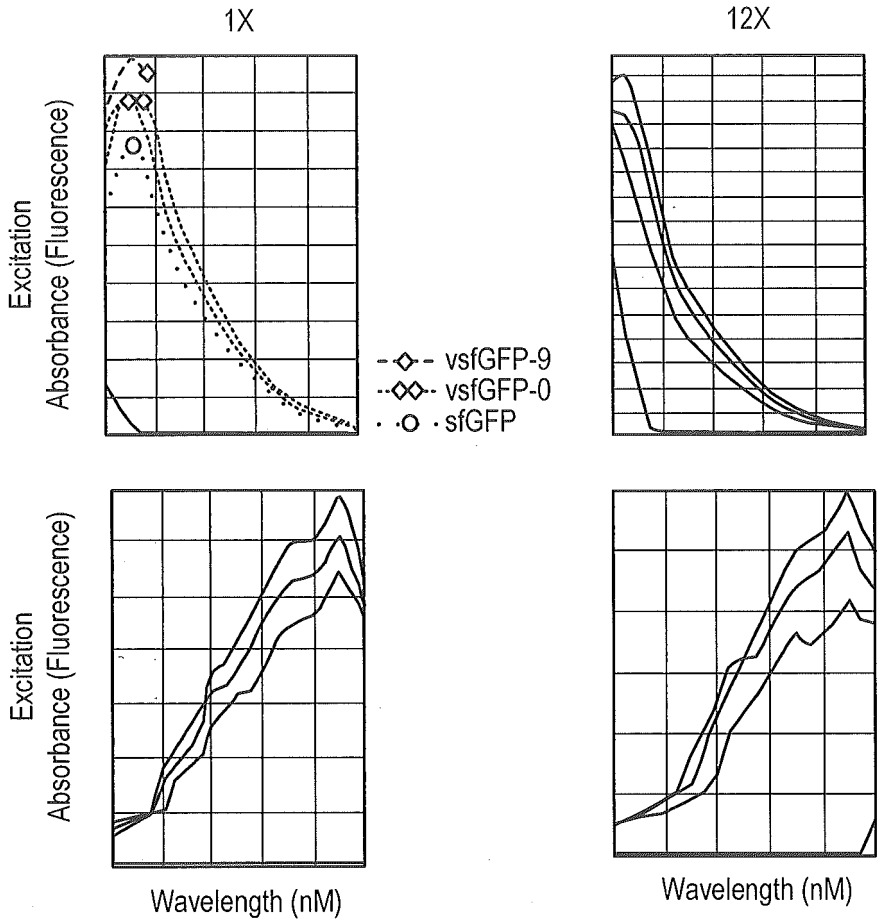


Figure 6



Protein	Calculated MW	ε	Slope	Brightness (per mole)	Relative brightness
sfGFP	27604	19035	91642	1.74E+09	1
vsfGFP-0	39210.9	44600	59056	2.63E+09	1.51
vsfGFP-9	40286.1	46090	59834	2.76E+09	1.58

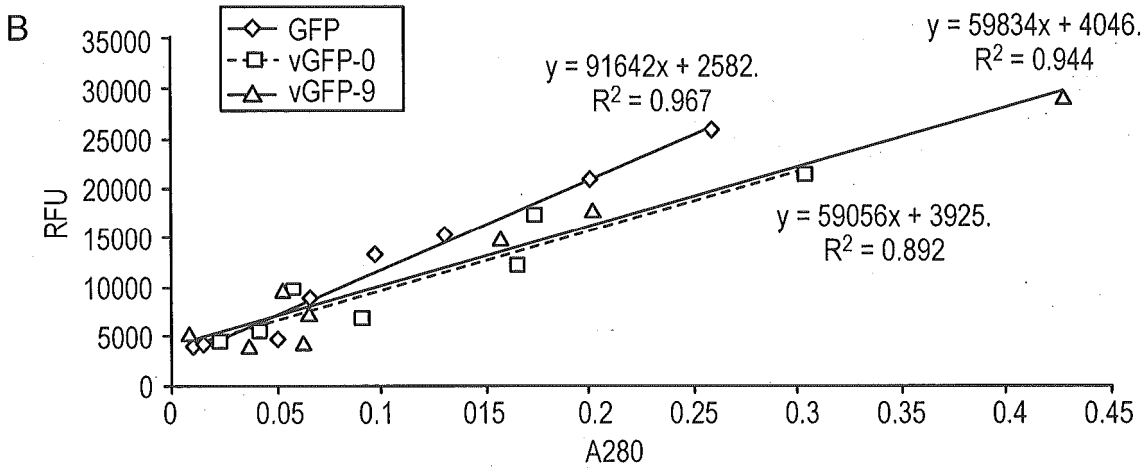


Figure 7

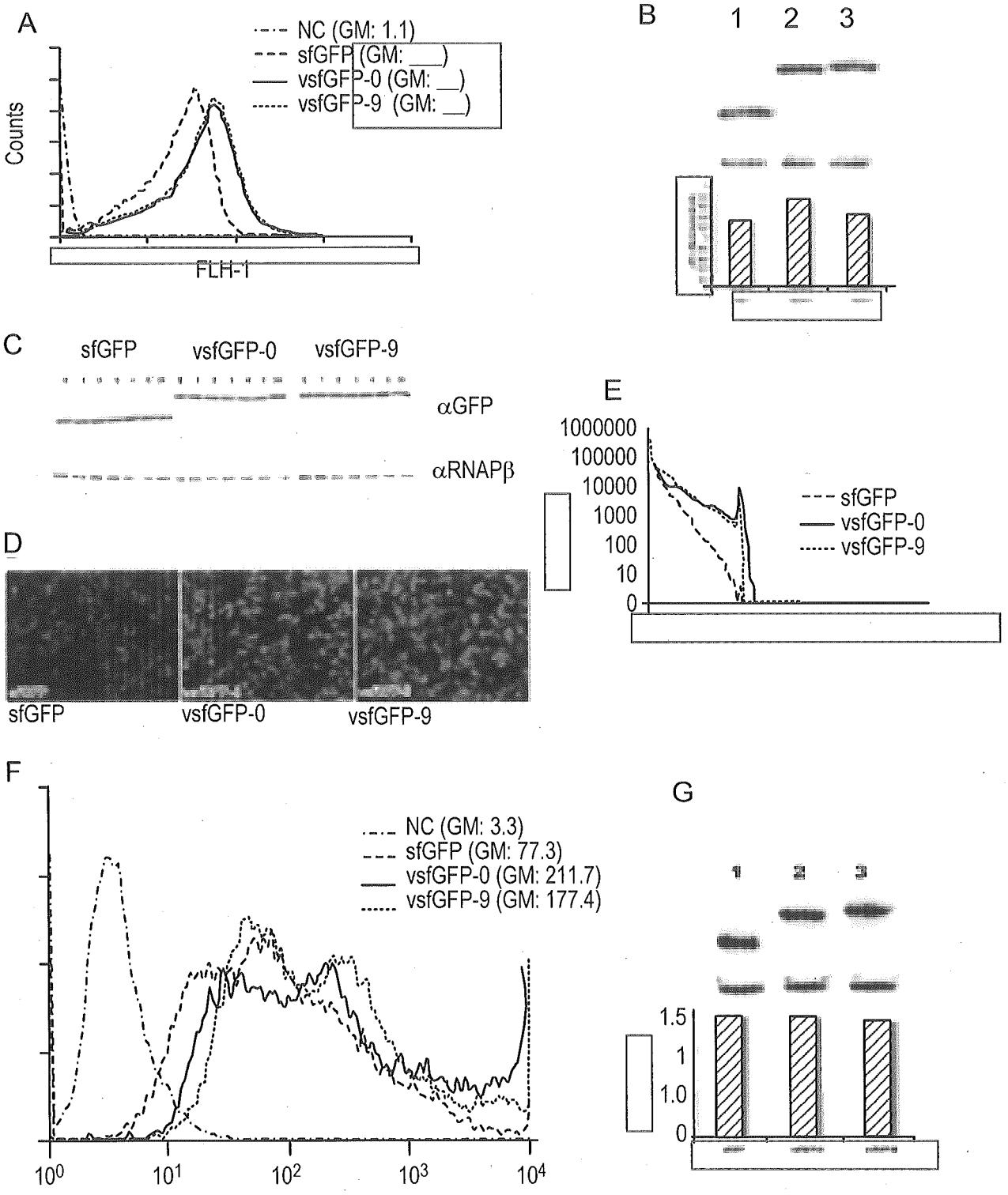

A
NC (GM: 1.1)
sfGFP (GM: ___)
vsfGFP-0 (GM: __)
vsfGFP-9 (GM: __)
Counts
FLH-1
B
1 2 3
C
sfGFP
vsfGFP-0
vsfGFP-9
αGFP
αRNAPβ
D
sfGFP
vsfGFP-0
vsfGFP-9
E
1000000
100000
10000
1000
100
10
0
sfGFP
vsfGFP-0
vsfGFP-9
F
NC (GM: 3.3)
sfGFP (GM: 77.3)
vsfGFP-0 (GM: 211.7)
vsfGFP-9 (GM: 177.4)
10^0
10^1
10^2
10^3
10^4
G
1 2 3
1.5
1
1.0
0

Figure 8

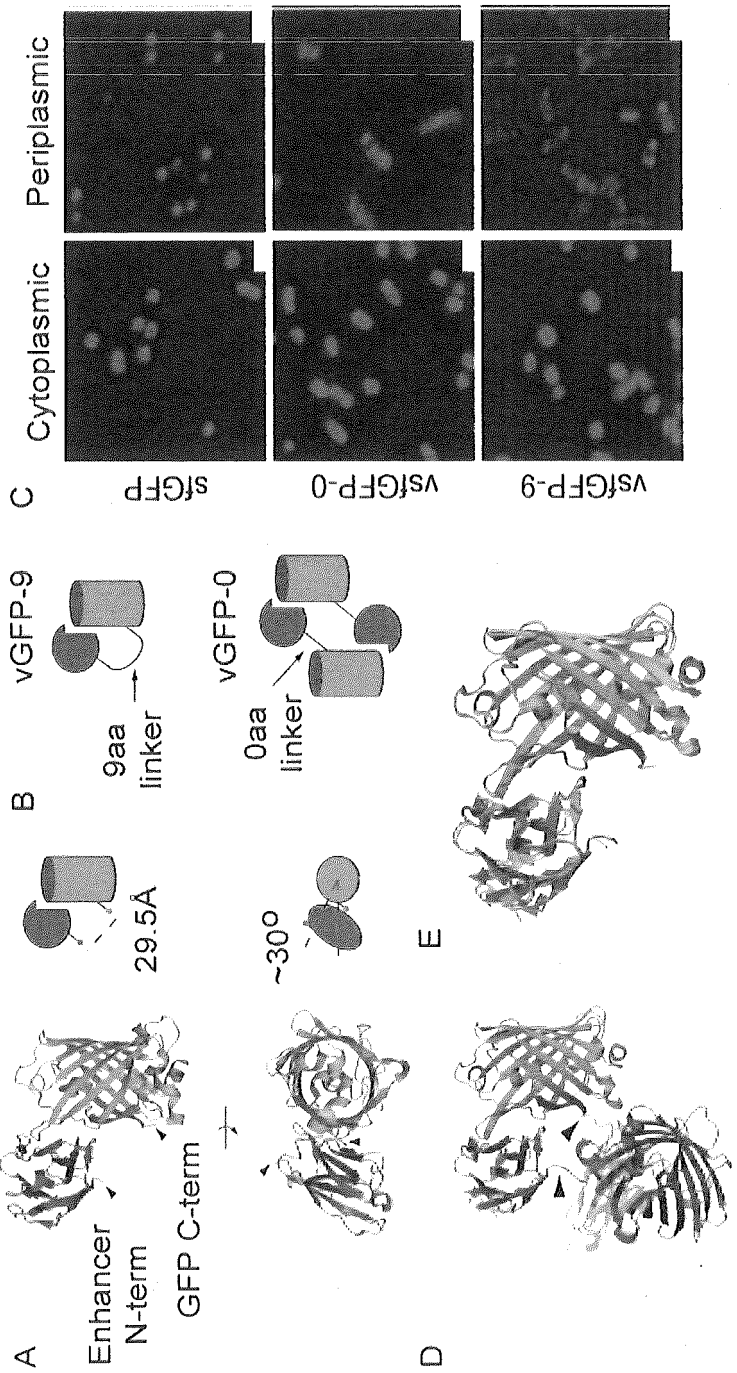

Periplasmic
Cytoplasmic
C
sfGFP
vsfGFP-0
vsfGFP-9
B
vGFP-9
9aa linker
vGFP-0
0aa linker
29.5Å
~30°
A
Enhancer N-term
GFP C-term
D
E

Figure 9

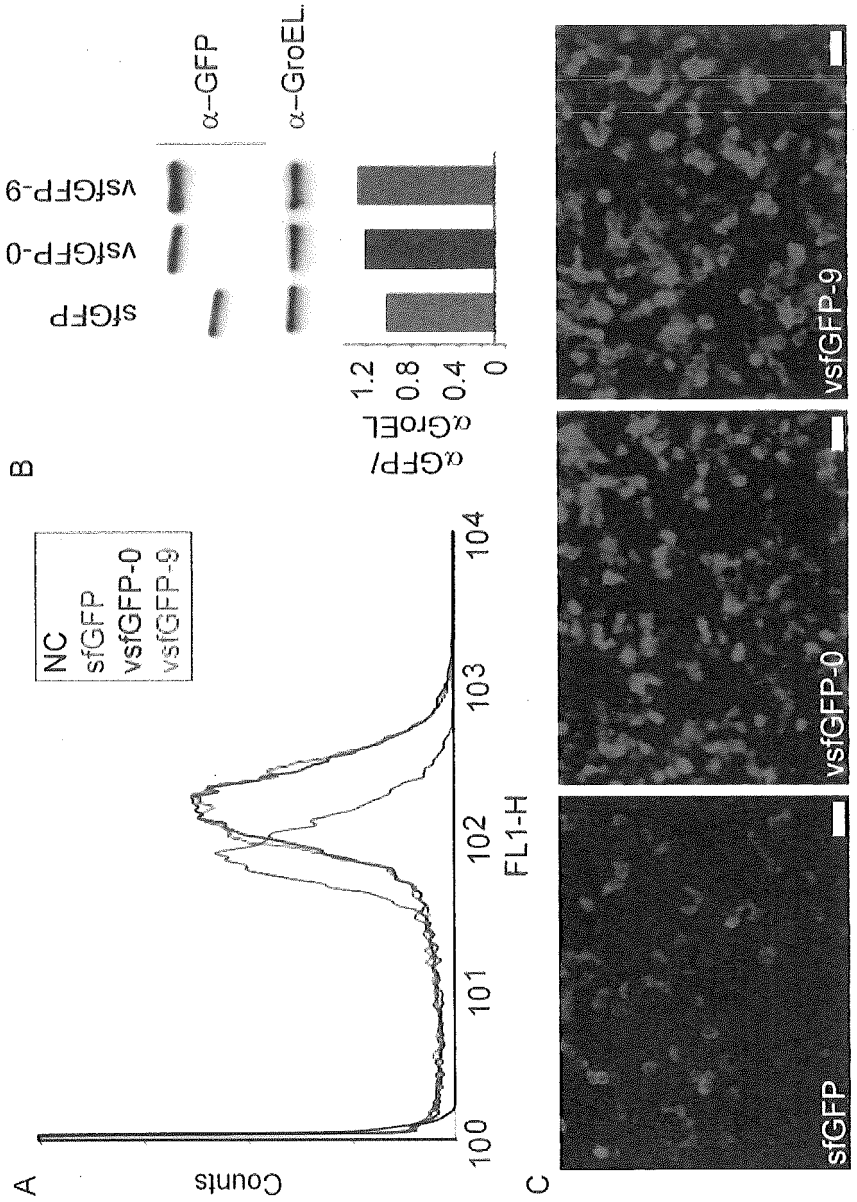

A
Counts
100
101
102
103
104
FL1-H
NC
sfGFP
vsfGFP-0
vsfGFP-9
B
α–GFP
α–GroEL
sfGFP
vsfGFP-0
vsfGFP-9
αGFP/
αGroEL
0
0.4
0.8
1.2
C
sfGFP
vsfGFP-0
vsfGFP-9

Figure 10

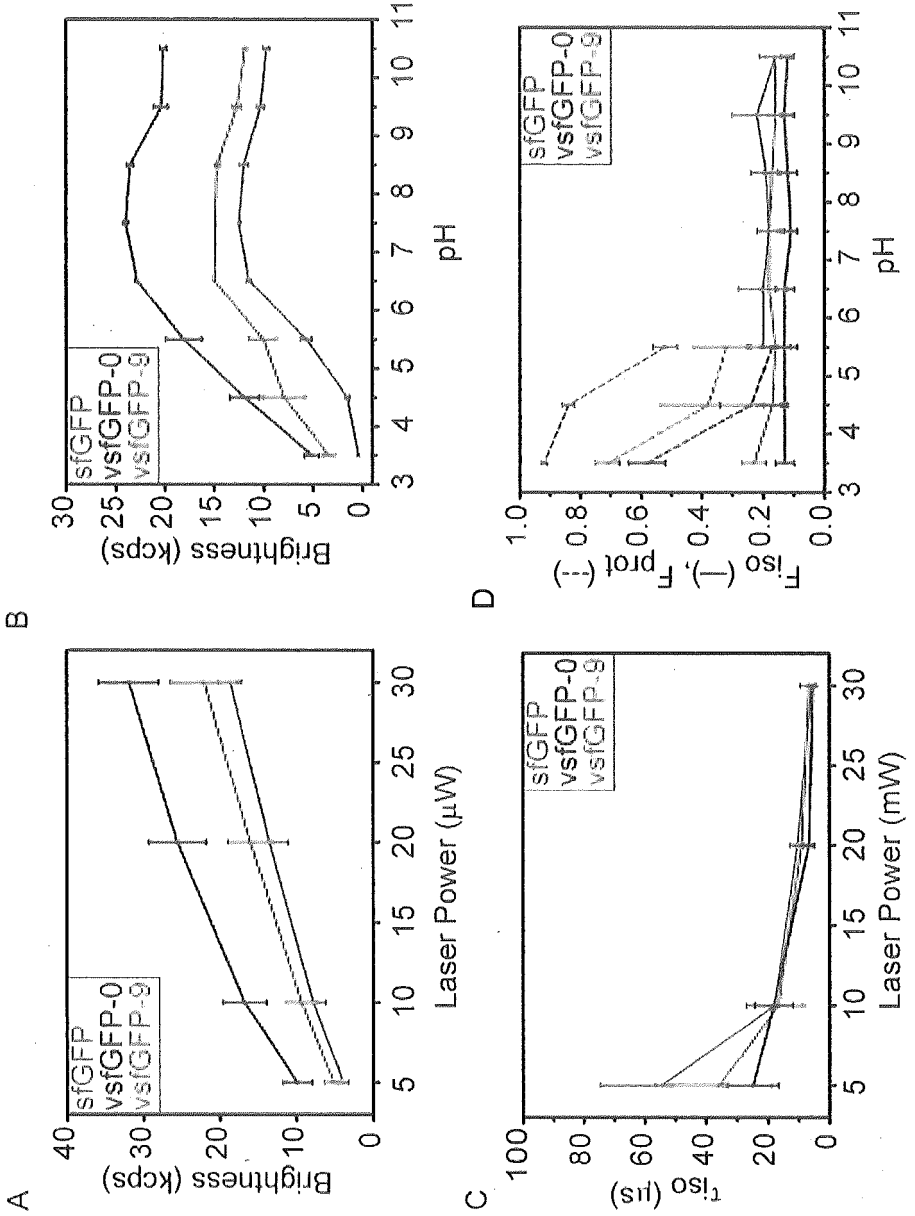

A
Brightness (kcps)
Laser Power (µW)
sfGFP
vsfGFP-0
vsfGFP-9
B
Brightness (kcps)
pH
C
τiso (µs)
Laser Power (mW)
D
Fiso (—), Fprot (- -)
pH

Figure 11

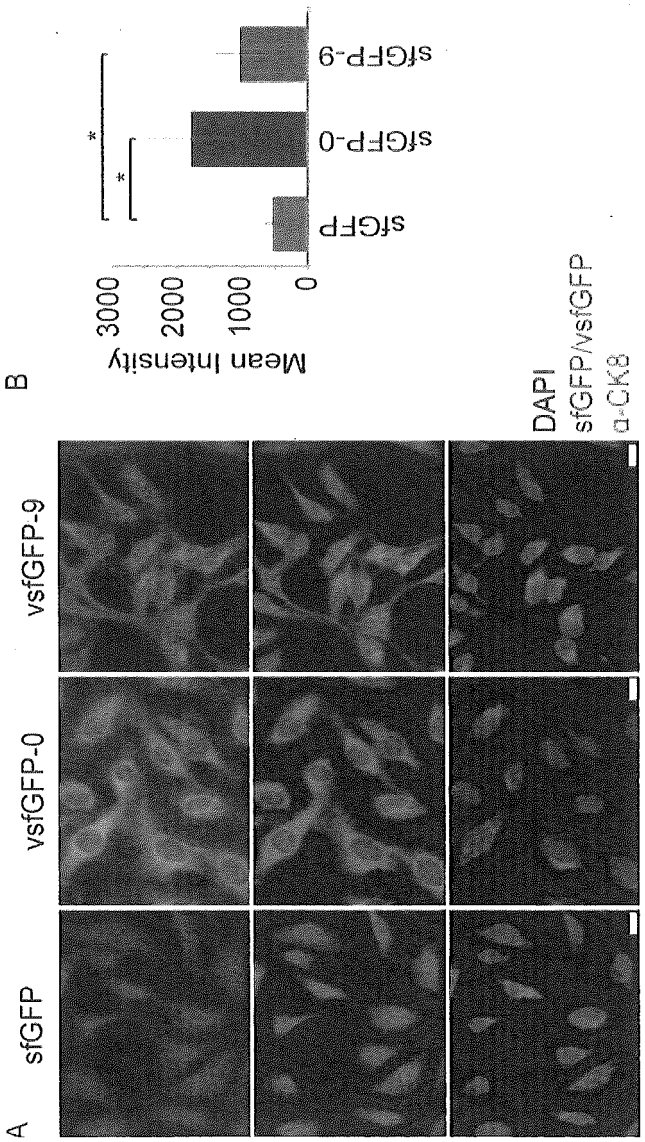

A
sfGFP
vsfGFP-0
vsfGFP-9
DAPI
sfGFP/vsfGFP
α-CK8
B
Mean Intensity
3000
2000
1000
0
sfGFP
sfGFP-0
sfGFP-9
*
*

Figure 12

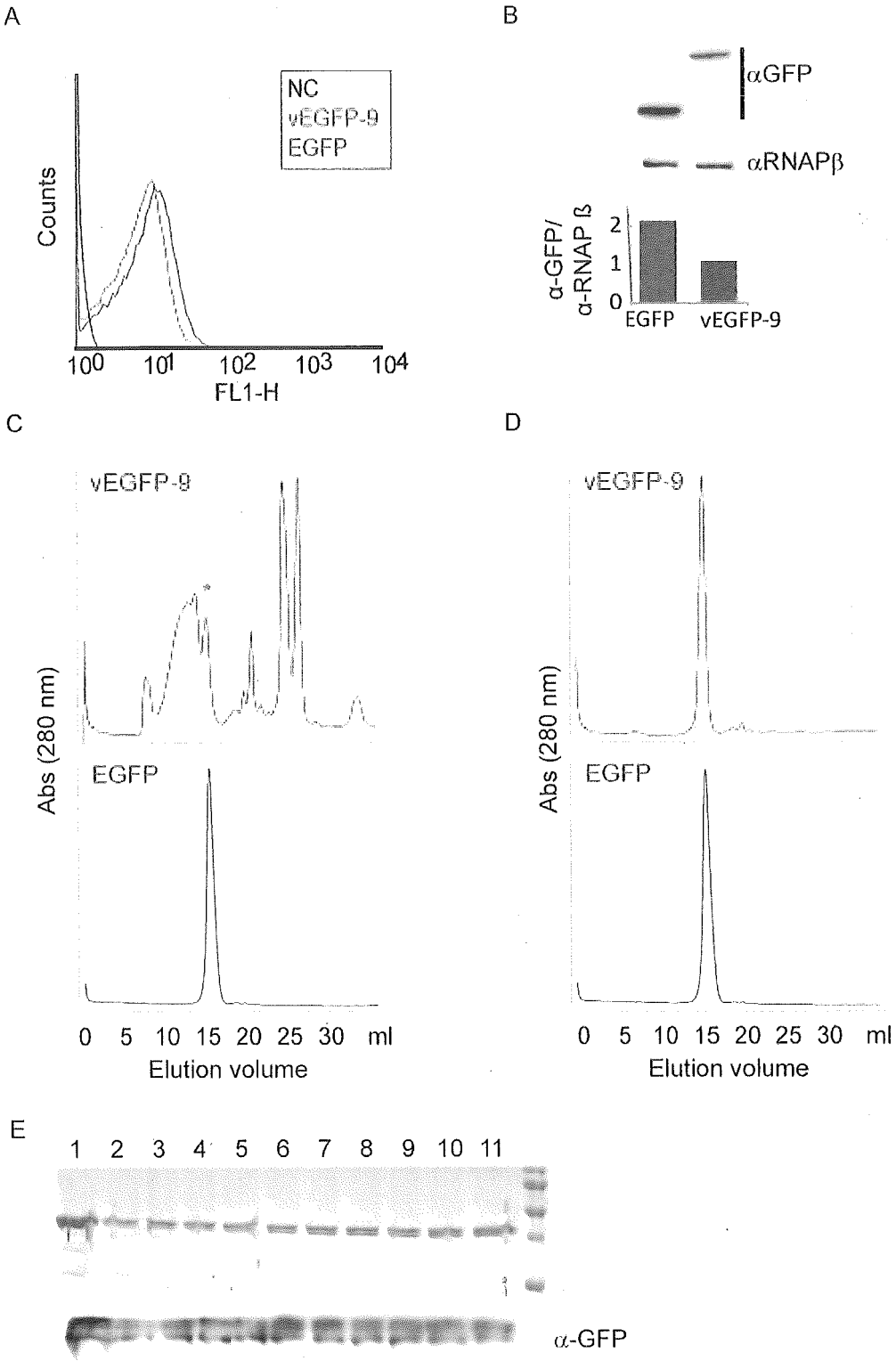

A
NC
vEGFP-9
EGFP
Counts
10^0 10^1 10^2 10^3 10^4
FL1-H
B
αGFP
αRNAPβ
α-GFP/ α-RNAP ß
2
1
0
EGFP
vEGFP-9
C
vEGFP-9
EGFP
Abs (280 nm)
0 5 10 15 20 25 30 ml
Elution volume
D
vEGFP-9
EGFP
Abs (280 nm)
0 5 10 15 20 25 30 ml
Elution volume
E
1 2 3 4 5 6 7 8 9 10 11
α-GFP

Figure 13

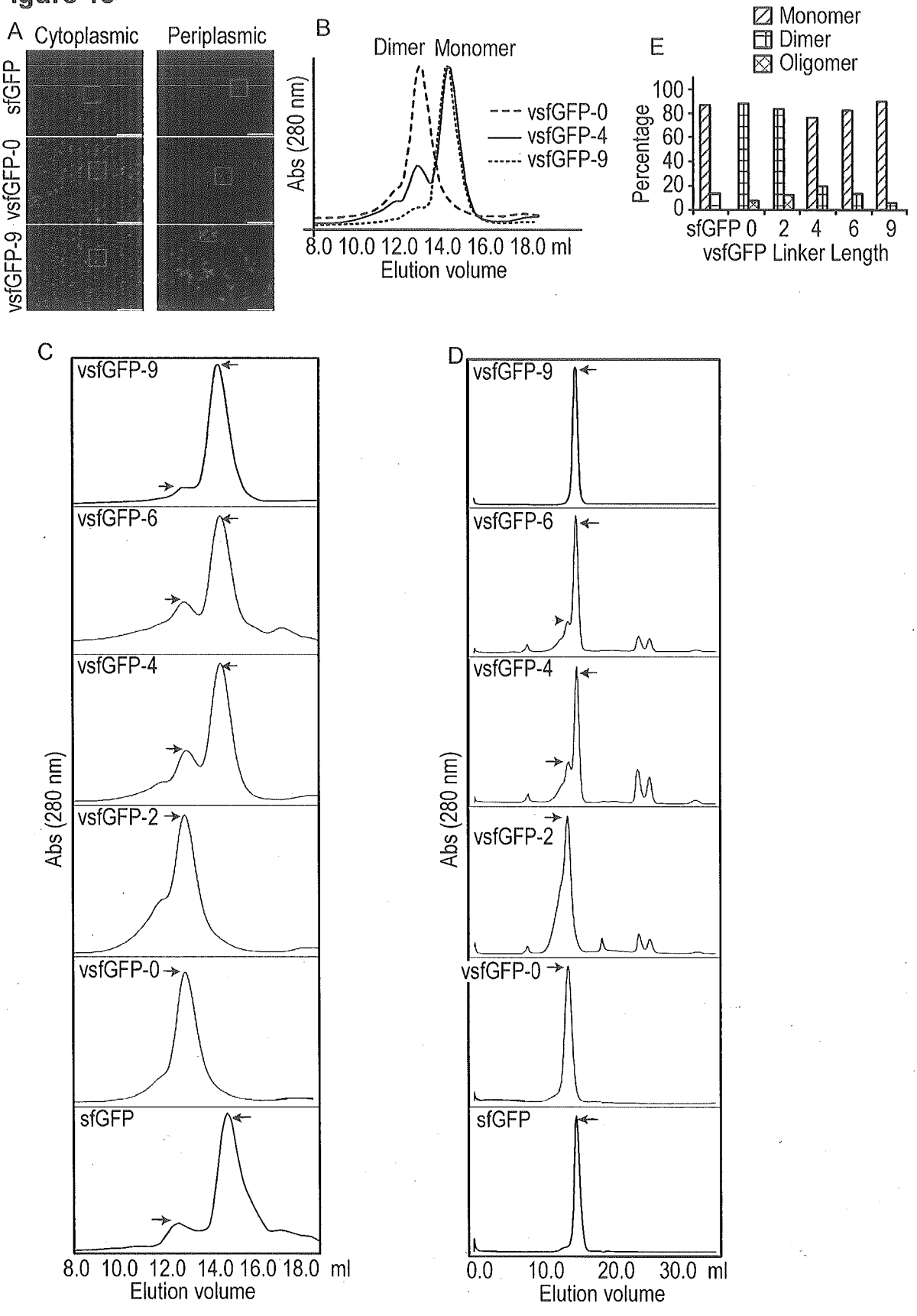

A
Cytoplasmic
Periplasmic
sfGFP
vsfGFP-0
vsfGFP-9
B
Dimer
Monomer
Abs (280 nm)
vsfGFP-0
vsfGFP-4
vsfGFP-9
8.0 10.0 12.0 14.0 16.0 18.0 ml
Elution volume
E
Monomer
Dimer
Oligomer
Percentage
100
80
60
40
20
0
sfGFP 0 2 4 6 9
vsfGFP Linker Length
C
vsfGFP-9
vsfGFP-6
vsfGFP-4
vsfGFP-2
vsfGFP-0
sfGFP
Abs (280 nm)
8.0 10.0 12.0 14.0 16.0 18.0 ml
Elution volume
D
vsfGFP-9
vsfGFP-6
vsfGFP-4
vsfGFP-2
vsfGFP-0
sfGFP
Abs (280 nm)
0.0 10.0 20.0 30.0 ml
Elution volume

Figure 14

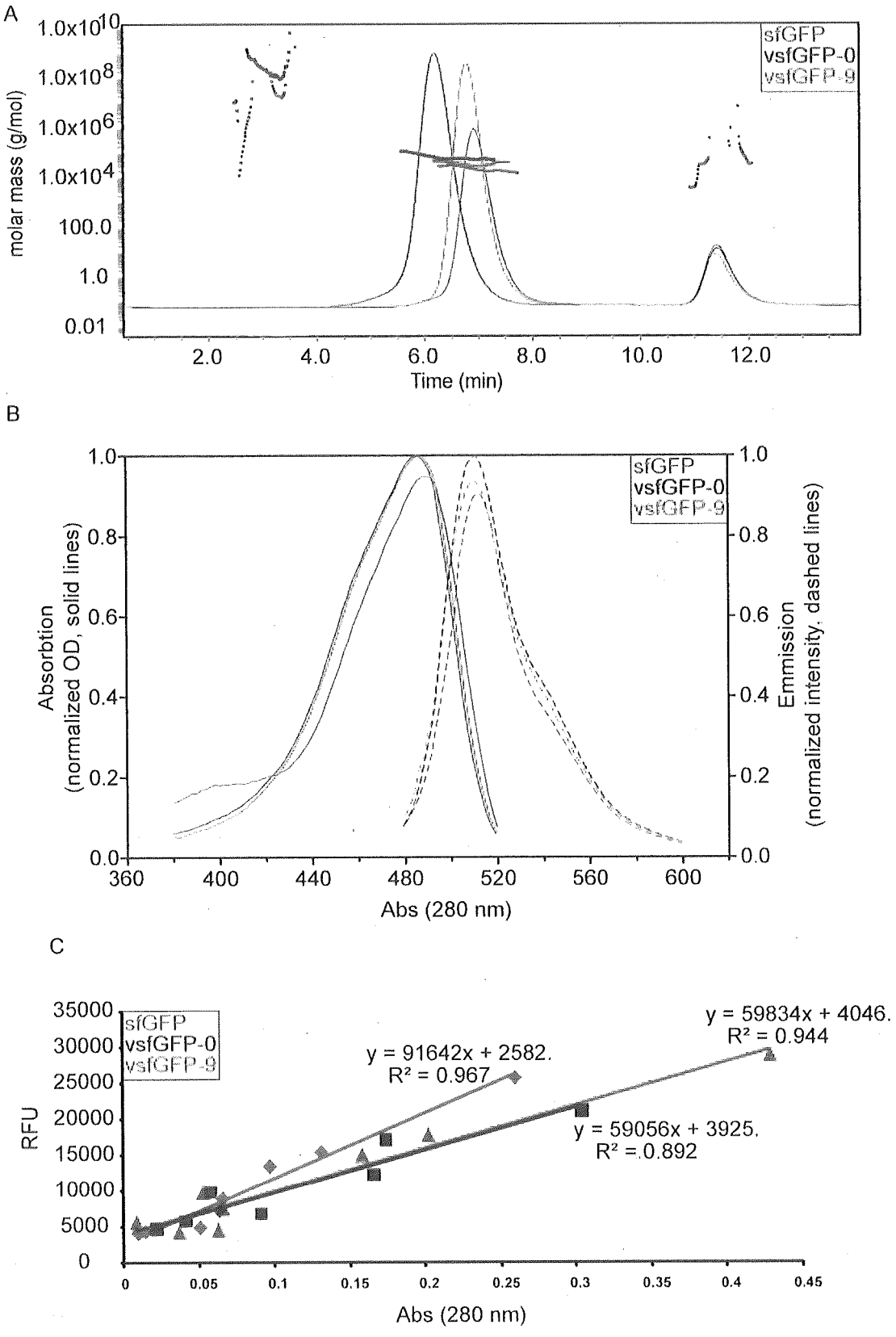
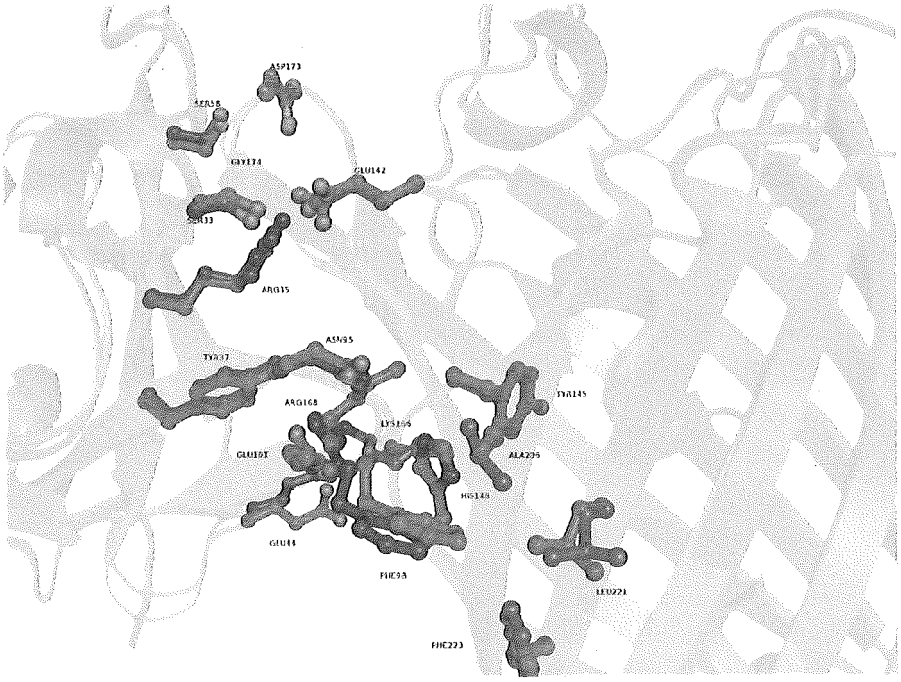

A
molar mass (g/mol)
1.0x10^10
1.0x10^8
1.0x10^6
1.0x10^4
100.0
1.0
0.01
2.0
4.0
6.0
8.0
10.0
12.0
Time (min)
sfGFP
vsfGFP-0
vsfGFP-9
B
Absorbtion (normalized OD, solid lines)
Emmission (normalized intensity, dashed lines)
0.0
0.2
0.4
0.6
0.8
1.0
360
400
440
480
520
560
600
Abs (280 nm)
sfGFP
vsfGFP-0
vsfGFP-9
C
RFU
35000
30000
25000
20000
15000
10000
5000
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
Abs (280 nm)
y = 91642x + 2582.
R² = 0.967
y = 59834x + 4046.
R² = 0.944
y = 59056x + 3925.
R² = 0.892
sfGFP
vsfGFP-0
vsfGFP-9

Figure 15

A


ASP173
GLY134
GLU142
ARG15
ASN35
TYR37
ARG168
GLU167
HIS148
GLU44
PHE93
LEU221
PHE223

B

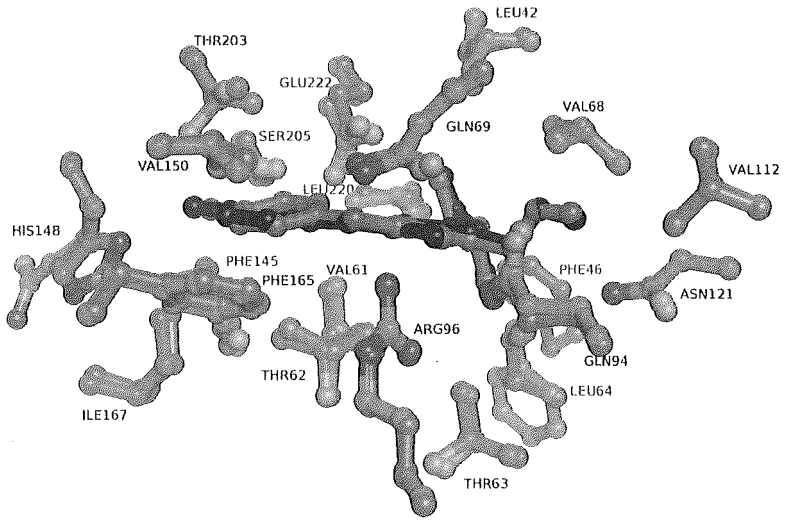

LEU42
THR203
GLU222
VAL68
GLN69
SER205
VAL150
VAL112
LEU220
HIS148
PHE145
PHE165
VAL61
PHE46
ASN121
ARG96
GLN94
THR62
LEU64
ILE167
THR63

Figure 16

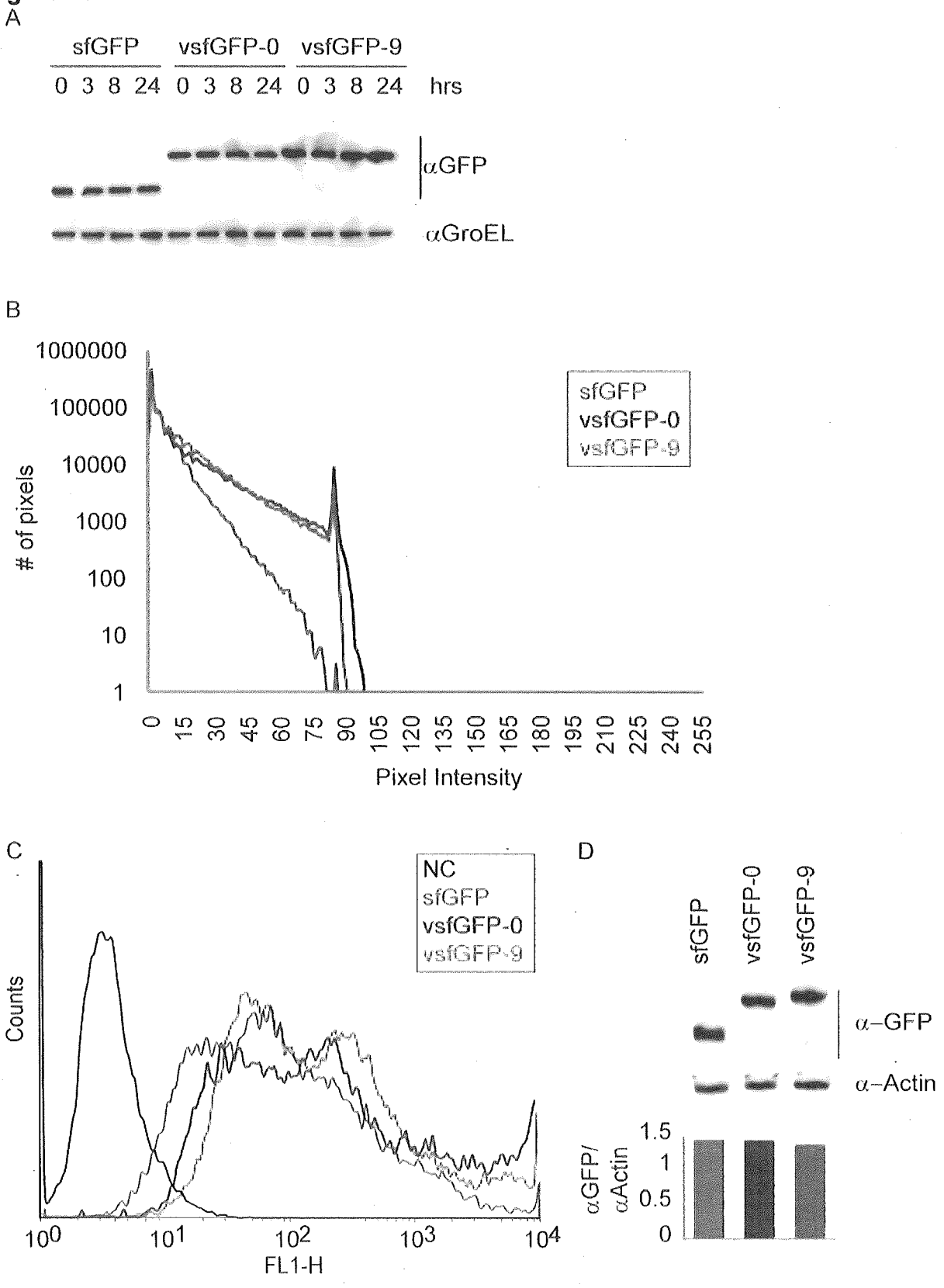

A
sfGFP
vsfGFP-0
vsfGFP-9
0 3 8 24 0 3 8 24 0 3 8 24 hrs
αGFP
αGroEL
B
1000000
100000
10000
1000
100
10
1
# of pixels
0 15 30 45 60 75 90 105 120 135 150 165 180 195 210 225 240 255
Pixel Intensity
sfGFP
vsfGFP-0
vsfGFP-9
C
Counts
NC
sfGFP
vsfGFP-0
vsfGFP-9
10^0 10^1 10^2 10^3 10^4
FL1-H
D
sfGFP
vsfGFP-0
vsfGFP-9
α–GFP
α–Actin
αGFP/ αActin
1.5
1
0.5
0

Figure 17

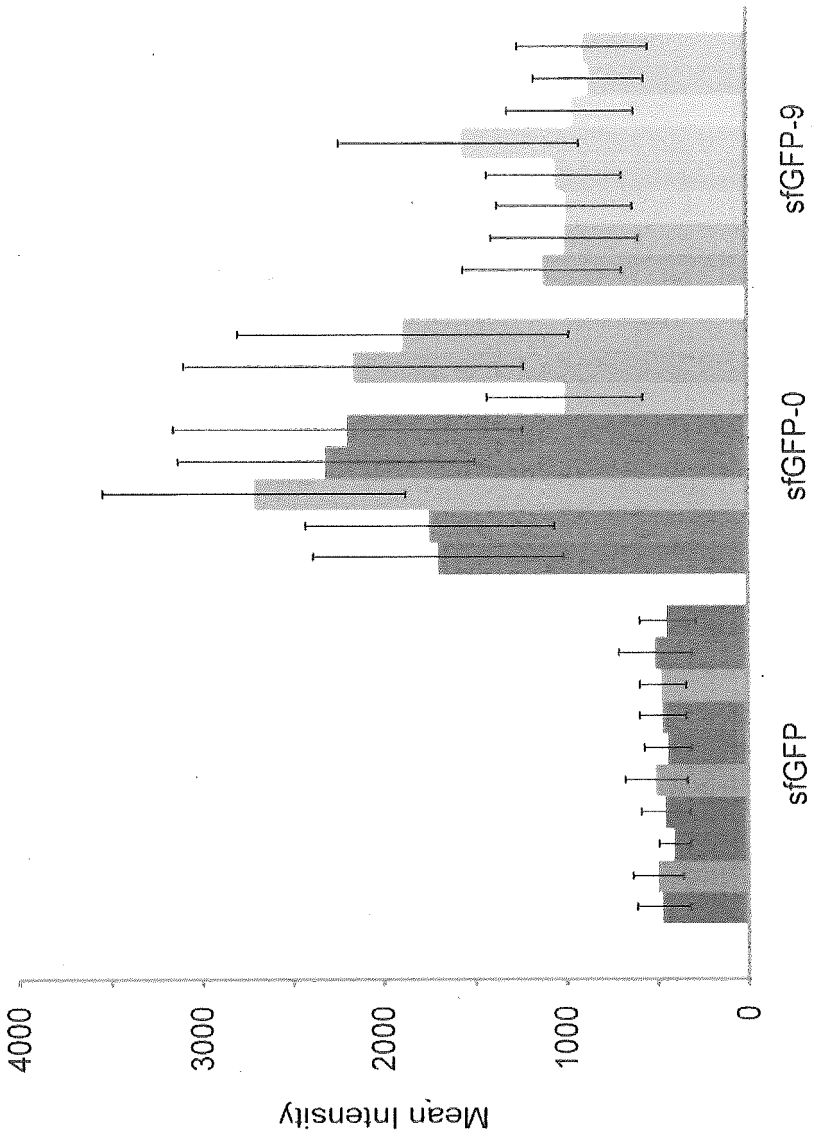


Figure 18A

```
Met Ala Asp Val Gln Leu Val Glu Ser Gly Gly Ala Leu Val Gln Pro
1               5                  10                  15

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Val Asn
            20                  25                  30

Arg Tyr Ser Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu
        35                  40                  45

Trp Val Ala Gly Met Ser Ser Ala Gly Asp Arg Ser Ser Tyr Glu Asp
    50                  55                  60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Arg Asn Thr
65                  70                  75                  80

Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Asn Val Asn Val Gly Phe Glu Tyr Trp Gly Gln Gly Thr Gln
            100                 105                 110

Val Thr Val Ser Ser Arg Asn Ser Ile Ser Ser Leu Ser Ile Pro Ser
        115                 120                 125

Thr Val Pro Arg Ala Arg Asp Pro Pro Val Asp Met Ala Ser Ser Glu
    130                 135                 140

Asp Val Ile Lys Glu Phe Met Arg Phe Lys Val Arg Met Glu Gly Ser
145                 150                 155                 160

Val Asn Gly His Glu Phe Glu Ile Glu Gly Glu Gly Glu Gly Arg Pro
```

Figure 18A (continued)

```
Tyr Glu Gly Thr Gln Thr Ala Lys Leu Lys Val Thr Lys Gly Gly Pro
            180                 185                 190

Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Phe Gln Tyr Gly Ser
        195                 200                 205

Lys Ala Tyr Val Lys His Pro Ala Asp Ile Pro Asp Tyr Leu Lys Leu
    210                 215                 220

Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val Met Asn Phe Glu Asp
225                 230                 235                 240

Gly Gly Val Val Thr Val Thr Gln Asp Ser Ser Leu Gln Asp Gly Glu
                245                 250                 255

Phe Ile Tyr Lys Val Lys Leu Arg Gly Thr Asn Phe Pro Ser Asp Gly
            260                 265                 270

Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu Ala Ser Thr Glu Arg
        275                 280                 285

Met Tyr Pro Glu Asp Gly Ala Leu Lys Gly Glu Ile Lys Met Arg Leu
    290                 295                 300

Lys Leu Lys Asp Gly Gly His Tyr Asp Ala Glu Val Lys Thr Thr Tyr
305                 310                 315                 320

Met Ala Lys Lys Pro Val Gln Leu Pro Gly Ala Tyr Lys Thr Asp Ile
                325                 330                 335

Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr Thr Ile Val Glu Gln
            340                 345                 350

Tyr Glu Arg Ala Glu Gly Arg His Ser Thr Gly Ala Gly Leu Tyr Lys
        355                 360                 365
```

Figure 18B

```
atggccgatg tgcagctgct ggagtctggg ggagccttgg tgcagccggg ggggtctctg      60
agactctcct gtgcagcctc tggattcccc gtcaatcgct atagtatggg gtggtaccgc     120
caggctccag ggaaggagcg cgagtgggtc gcgggtatga gtagtgctgg tgatcgttca     180
agttatgaag actccgtgaa gggccgattc accatctcca gagacgacgc caggaatacg     240
gtgtatctgc aaatgaacag cctgaaacct gaggacacgg ccgtgtatta ctgtaatgtc     300
aatgtgggct ttgagtactg gggccagggg acccaggtca ccgtctcctc aaggaattcg     360
atatcaagct tatcgatacc gtcgacggta ccgcgggccc gggatccacc ggtcgacatg     420
gcctcctccg aggacgtcat caaggagttc atgcgcttca aggtgcgcat ggagggctcc     480
gtgaacggcc acgagttcga gatcgagggc gagggcgagg gccgccccta cgagggcacc     540
cagaccgcca agctgaaggt gaccaagggc ggccccctgc ccttcgcctg ggacatcctg     600
tcccctcagt tccagtacgg ctccaaggcc tacgtgaagc accccgccga catccccgac     660
tacttgaagc tgtccttccc cgagggcttc aagtgggagc gcgtgatgaa cttcgaggac     720
ggcggcgtgg tgaccgtgac ccaggactcc tccctgcagg acggcgagtt catctacaag     780
gtgaagctgc gcggcaccaa cttcccctcc gacggccccg taatgcagaa gaagaccatg     840
ggctgggagg cctccaccga gcggatgtac cccgaggacg gcgccctgaa gggcgagatc     900
aagatgaggc tgaagctgaa ggacggcggc cactacgacg ccgaggtcaa gaccacctac     960
atggccaaga agcccgtgca gctgcccggc gcctacaaga ccgacatcaa gctggacatc    1020
acctccccaca acgaggacta caccatcgtg gaacagtacg agcgcgccga gggccgccac    1080
tccaccggcg ccggcctgta caagtaa                                        1107
```

Figure 19A

```
Met Ala Asp Val Gln Leu Val Glu Ser Gly Gly Ala Leu Val Gln Pro
1               5                  10                  15

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Val Asn
            20                  25                  30

Arg Tyr Ser Met Arg Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu
        35                  40                  45

Trp Val Ala Gly Met Ser Ser Ala Gly Asp Arg Ser Ser Tyr Glu Asp
    50                  55                  60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Arg Asn Thr
65                  70                  75                  80

Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Ser Asn Val Asn Val Gly Phe Glu Tyr Trp Gly Gln Gly Thr Gln
            100                 105                 110

Val Thr Val Ser Ser Arg Asn Ser Ile Ser Ser Leu Ser Ile Pro Ser
        115                 120                 125

Thr Val Pro Arg Ala Arg Asp Pro Pro Val Asp Met Ala Ser Ser Glu
    130                 135                 140

Asp Val Ile Lys Glu Phe Met Arg Phe Lys Val Arg Met Glu Gly Ser
145                 150                 155                 160

Val Asn Gly His Glu Phe Glu Ile Glu Gly Glu Gly Glu Gly Arg Pro
                165                 170                 175

Tyr Glu Gly Thr Gln Thr Ala Lys Leu Lys Val Thr Lys Gly Gly Pro
            180                 185                 190

Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Phe Gln Tyr Gly Ser
        195                 200                 205

Lys Ala Tyr Val Lys His Pro Ala Asp Ile Pro Asp Tyr Leu Lys Leu
    210                 215                 220
```

Figure 19A (continued)

```
Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val Met Asn Phe Glu Asp
225                 230                 235                 240

Gly Gly Val Val Thr Val Thr Gln Asp Ser Ser Leu Gln Asp Gly Glu
                245                 250                 255

Phe Ile Tyr Lys Val Lys Leu Arg Gly Thr Asn Phe Pro Ser Asp Gly
            260                 265                 270

Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu Ala Ser Thr Glu Arg
        275                 280                 285

Met Tyr Pro Glu Asp Gly Ala Leu Lys Gly Glu Ile Lys Met Arg Leu
    290                 295                 300

Lys Leu Lys Asp Gly Gly His Tyr Asp Ala Glu Val Lys Thr Thr Tyr
305                 310                 315                 320

Met Ala Lys Lys Pro Val Gln Leu Pro Gly Ala Tyr Lys Thr Asp Ile
                325                 330                 335

Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr Thr Ile Val Glu Gln
            340                 345                 350

Tyr Glu Arg Ala Glu Gly Arg His Ser Thr Gly Ala Gly Leu Tyr Lys
        355                 360                 365
```

Figure 19B

```
atggccgatg tgcagctggt ggagtctggg ggagccttgg tgcagccggg ggggtctctg     60
agactctcct gtgcagcctc tggattcccc gtcaatcgct atagtatgag gtggtaccgc    120
caggctccag ggaaggagcg cgagtgggtc gcgggtatga gtagtgctgg tgatcgttca    180
agttatgaag actccgtgaa gggccgattc accatctcca gagacgacgc caggaatacg    240
gtgtatctgc aaatgaacag cctgaaacct gaggacacgg ccgtgtatta ctcgaatgtc    300
aatgtgggct ttgagtactg gggccagggg acccaggtca ccgtctcctc aaggaattcg    360
atatcaagct tatcgatacc gtcgacggta ccgcgggccc gggatccacc ggtcgacatg    420
gcctcctccg aggacgtcat caaggagttc atgcgcttca aggtgcgcat ggagggctcc    480
gtgaacggcc acgagttcga gatcgagggc gagggcgagg gccgccccta cgagggcacc    540
cagaccgcca agctgaaggt gaccaagggc ggccccctgc ccttcgcctg ggacatcctg    600
tcccctcagt tccagtacgg ctccaaggcc tacgtgaagc accccgccga catccccgac    660
tacttgaagc tgtccttccc cgagggcttc aagtgggagc gcgtgatgaa cttcgaggac    720
ggcggcgtgg tgaccgtgac ccaggactcc tccctgcagg acggcgagtt catctacaag    780
gtgaagctgc gcggcaccaa cttccctcc gacggccccg taatgcagaa gaagaccatg    840
ggctgggagg cctccaccga gcggatgtac cccgaggacg gcgccctgaa gggcgagatc    900
aagatgaggc tgaagctgaa ggacggcggc cactacgacg ccgaggtcaa gaccacctac    960
atggccaaga agcccgtgca gctgcccggc gcctacaaga ccgacatcaa gctggacatc   1020
acctcccaca acgaggacta caccatcgtg gaacagtacg agcgcgccga gggccgccac   1080
tccaccggcg ccggcctgta caagtaa                                       1107
```

Figure 20

M A Q V Q L Q E S G G G L V
Q A G G S L T L S C T Y S G L T F D D Y
N M G W F R Q G P G K E R E R V S A I S
F R G I T Y Y Y V D S V K G R F T I S R D
N A K K T L Y L Q M N G L T P D D T A T
Y Y C A G S R F L S P F V R D G D T K L
I N D W G Q G T Q V T V S S