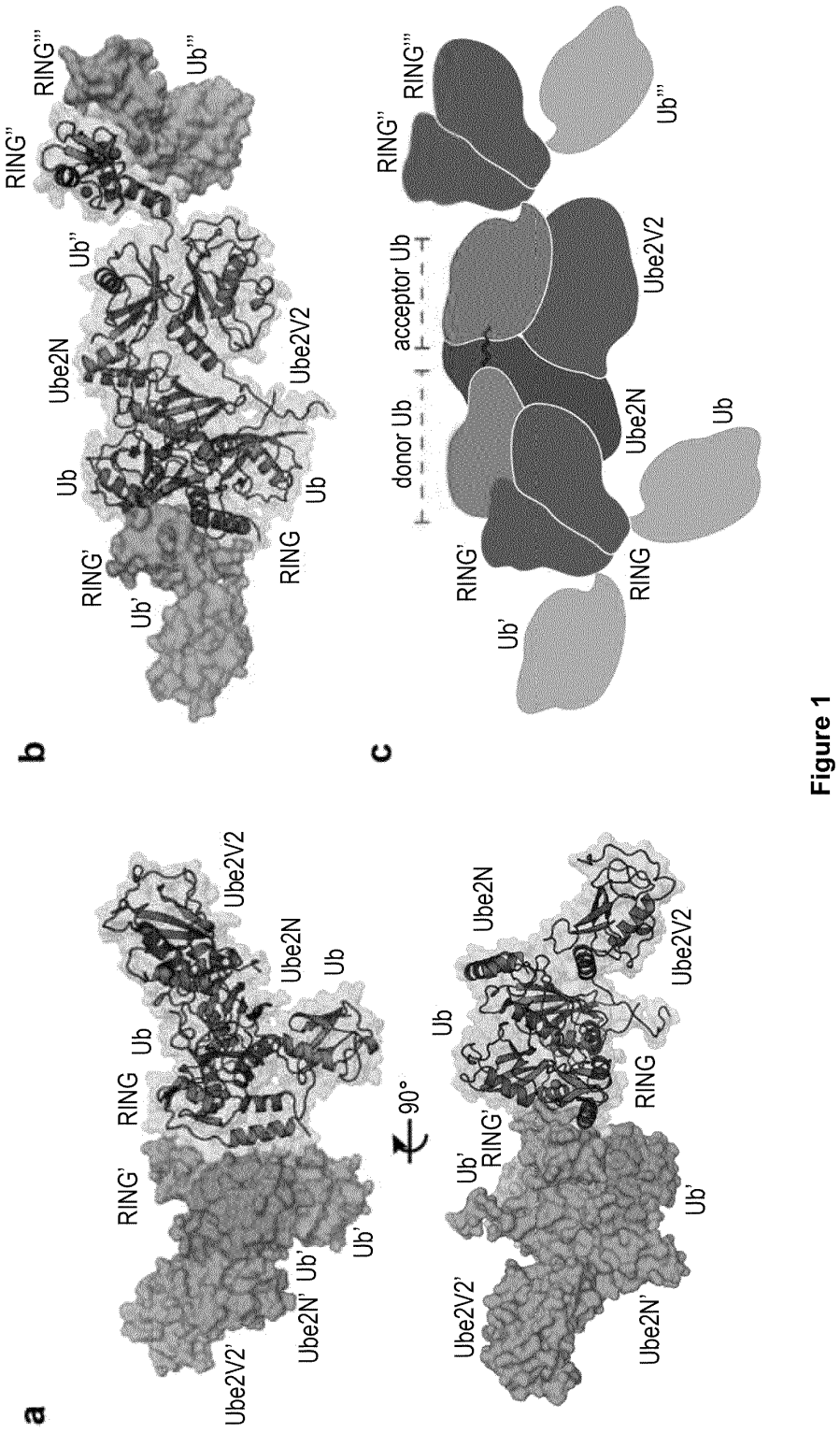

a
RING'
RING
Ub
Ube2V2
Ube2N
Ub
Ube2V2'
Ube2N'
Ub'
Ub'
90°
Ub
Ube2N
Ube2V2
RING'
Ub'
RING
Ube2V2'
Ube2N'
Ub'
b
RING''
RING'''
Ub'''
Ub''
Ube2N
Ube2V2
Ub
Ub
RING'
RING
Ub'
c
RING''
RING'''
Ub'''
donor Ub
acceptor Ub
Ube2V2
Ube2N
Ub
RING'
RING
Ub'

Figure 1

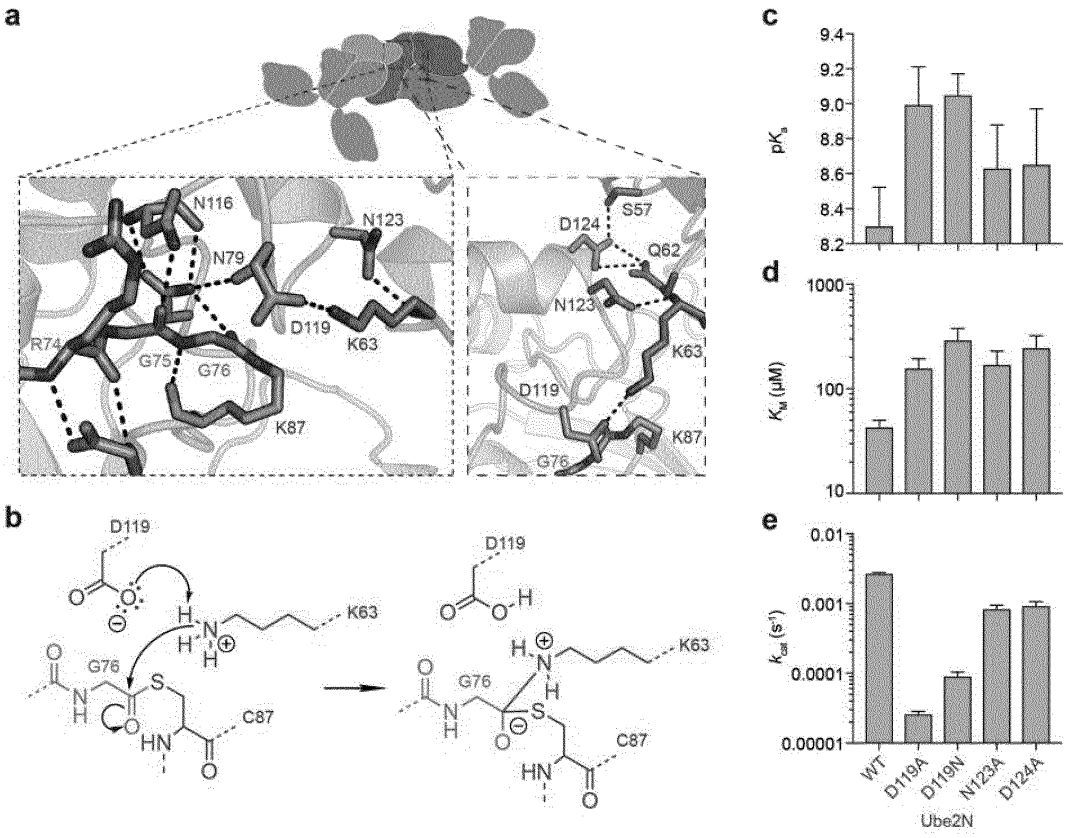

a
N116
N123
N79
D119
K63
R74
G75
G76
K87
S57
D124
Q62
N123
K63
D119
K87
G76
b
D119
K63
G76
C87
c
9.4
9.2
9.0
8.8
8.6
8.4
8.2
pKa
d
1000
100
10
KM (μM)
e
0.01
0.001
0.0001
0.00001
kcat (s-1)
WT
D119A
D119N
N123A
D124A
Ube2N

Figure 2

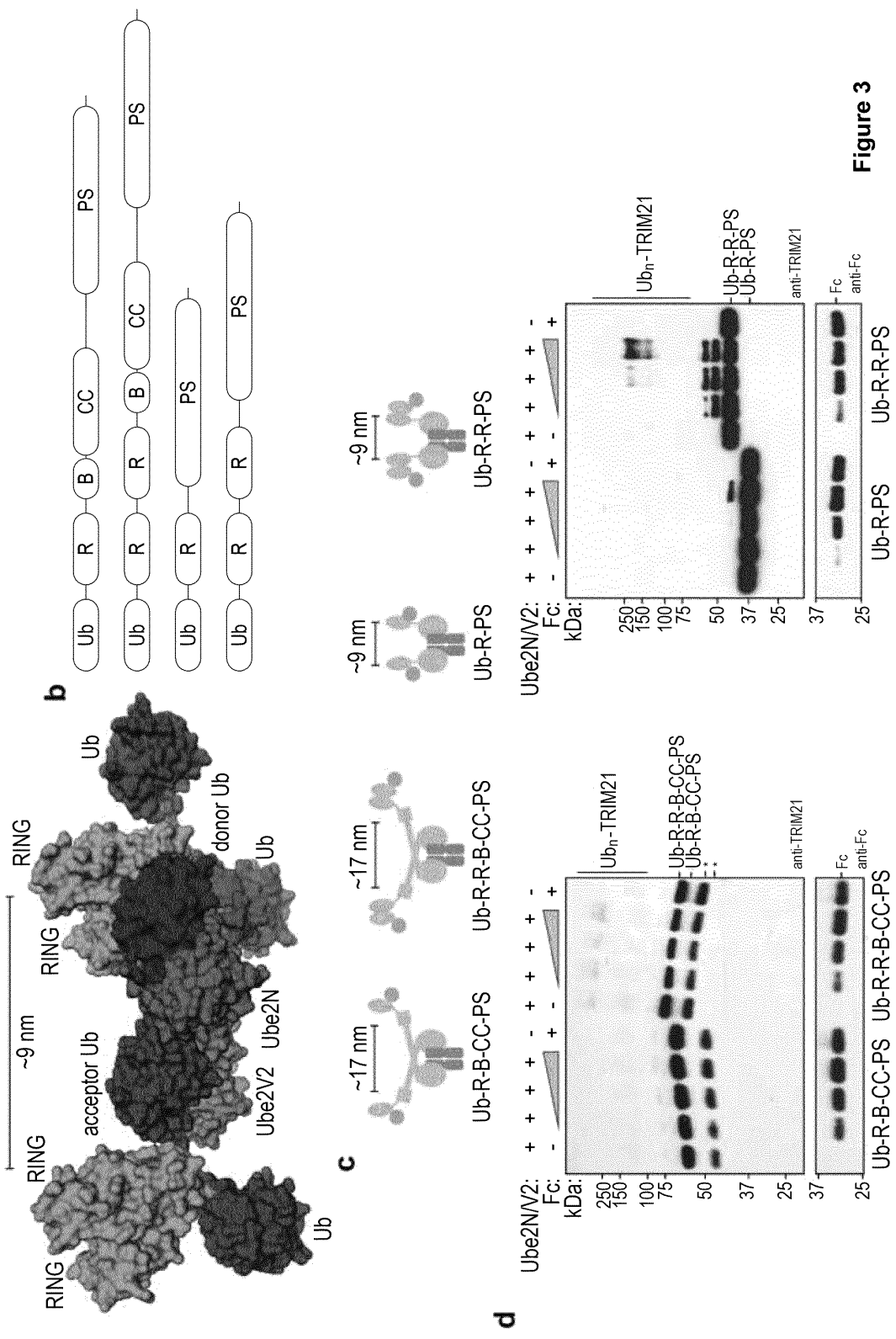

a
RING
RING
~9 nm
RING
RING
acceptor Ub
Ub
donor Ub
Ub
Ube2V2
Ube2N
Ub
b
Ub
R
B
CC
PS
Ub
R
R
B
CC
PS
Ub
R
PS
Ub
R
R
PS
c
~17 nm
Ub-R-B-CC-PS
~17 nm
Ub-R-R-B-CC-PS
~9 nm
Ub-R-PS
~9 nm
Ub-R-R-PS
d
Ube2N/V2:
Fc:
kDa:
250
150
100
75
50
37
25
Ub-R-B-CC-PS
Ub-R-R-B-CC-PS
Ub$_n$-TRIM21
Ub-R-B-CC-PS
Ub-R-R-B-CC-PS
anti-TRIM21
Fc
anti-Fc
Ub-R-PS
Ub-R-R-PS
Ub$_n$-TRIM21
Ub-R-R-PS
Ub-R-PS
anti-TRIM21
Fc
anti-Fc
Ub-R-PS
Ub-R-R-PS

Figure 3

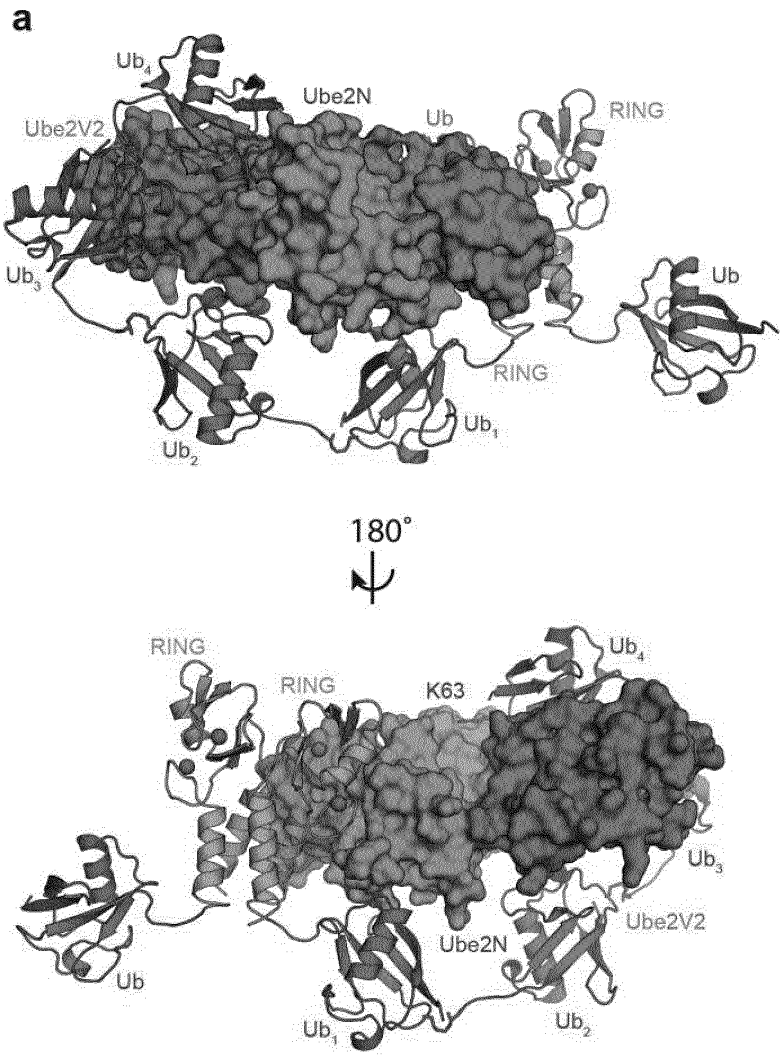

a
Ub4
Ube2N
Ube2V2
Ub
RING
Ub3
Ub
RING
Ub1
Ub2
180°
RING
RING
K63
Ub4
Ub3
Ube2V2
Ube2N
Ub
Ub1
Ub2

Figure 4A

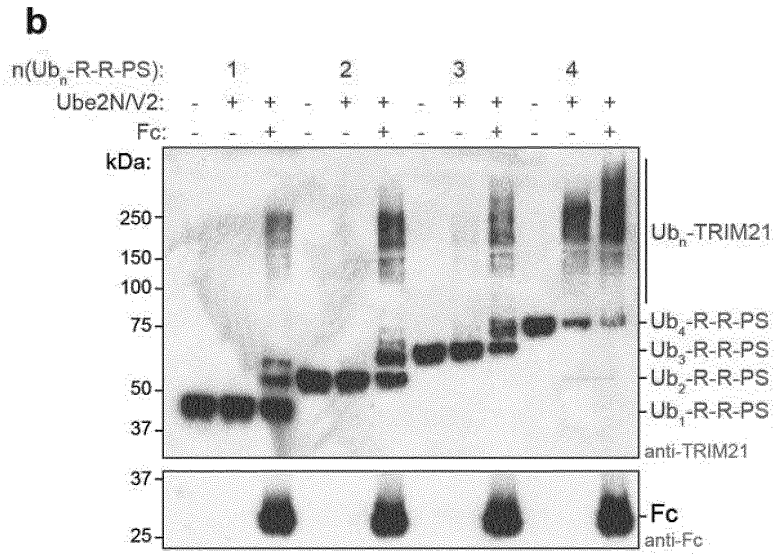

b
n(Ub$_n$-R-R-PS): 1 2 3 4
Ube2N/V2: - + + - + + - + + - + +
Fc: - - + - - + - - + - - +
kDa:
250
150
100
75
50
37
37
25
Ub$_n$-TRIM21
Ub$_4$-R-R-PS
Ub$_3$-R-R-PS
Ub$_2$-R-R-PS
Ub$_1$-R-R-PS
anti-TRIM21
Fc
anti-Fc

Figure 4B

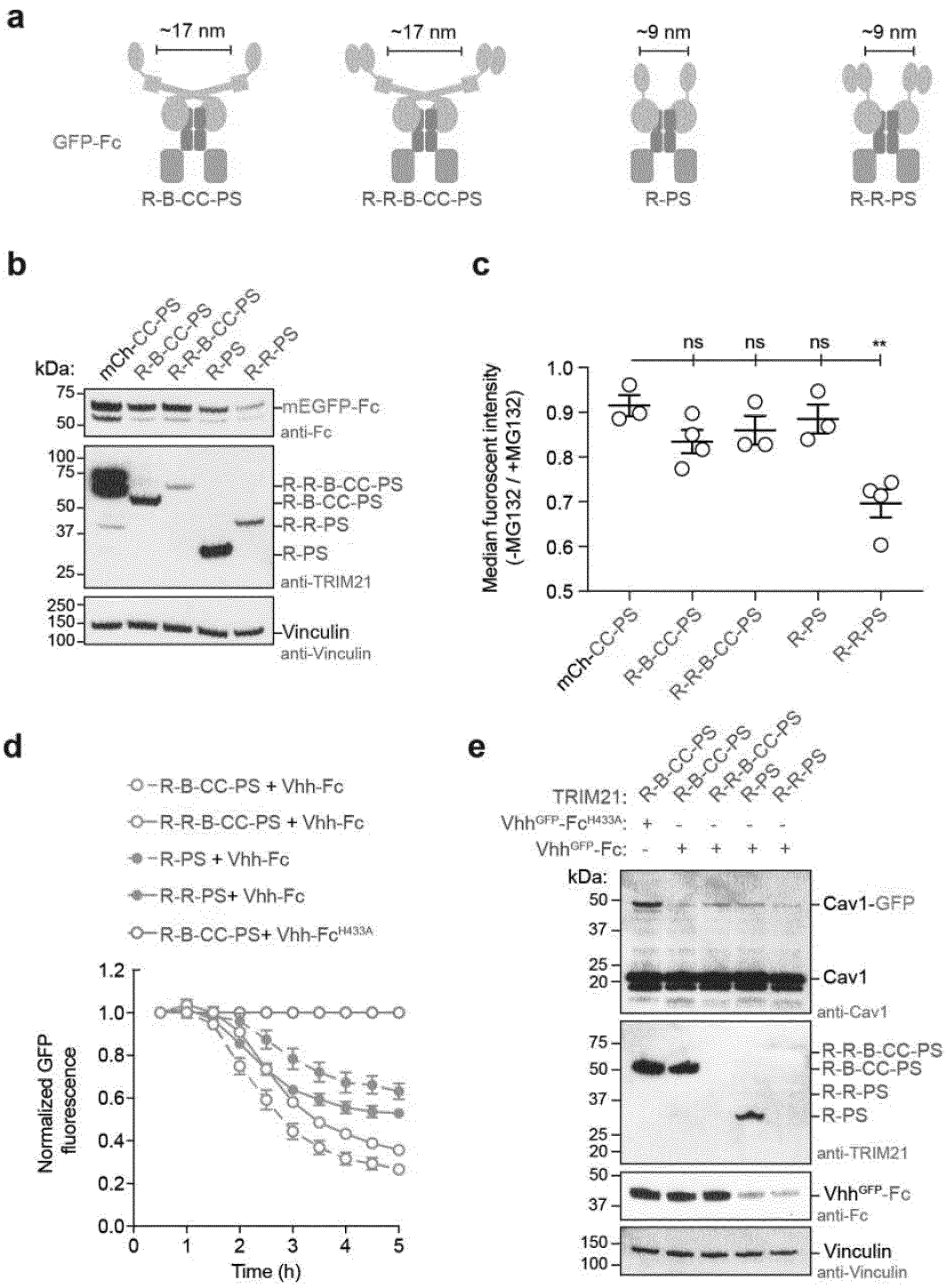

a
~17 nm
~17 nm
~9 nm
~9 nm
GFP-Fc
R-B-CC-PS
R-R-B-CC-PS
R-PS
R-R-PS
b
kDa:
mCh-CC-PS
R-B-CC-PS
R-R-B-CC-PS
R-PS
R-R-PS
75
50
mEGFP-Fc
anti-Fc
100
75
50
37
25
R-R-B-CC-PS
R-B-CC-PS
R-R-PS
R-PS
anti-TRIM21
250
150
100
Vinculin
anti-Vinculin
c
ns
ns
ns
**
Median fuoroscent intensity (-MG132 / +MG132)
1.0
0.9
0.8
0.7
0.6
0.5
mCh-CC-PS
R-B-CC-PS
R-R-B-CC-PS
R-PS
R-R-PS
d
R-B-CC-PS + Vhh-Fc
R-R-B-CC-PS + Vhh-Fc
R-PS + Vhh-Fc
R-R-PS+ Vhh-Fc
R-B-CC-PS+ Vhh-FcH433A
Normalized GFP fluorescence
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
Time (h)
e
TRIM21:
R-B-CC-PS
R-B-CC-PS
R-R-B-CC-PS
R-PS
R-R-PS
VhhGFP-FcH433A: + - - - -
VhhGFP-Fc: - + + + +
kDa:
50
37
25
20
Cav1-GFP
Cav1
anti-Cav1
75
50
37
25
20
R-R-B-CC-PS
R-B-CC-PS
R-R-PS
R-PS
anti-TRIM21
50
37
VhhGFP-Fc
anti-Fc
150
100
Vinculin
anti-Vinculin

Figure 5

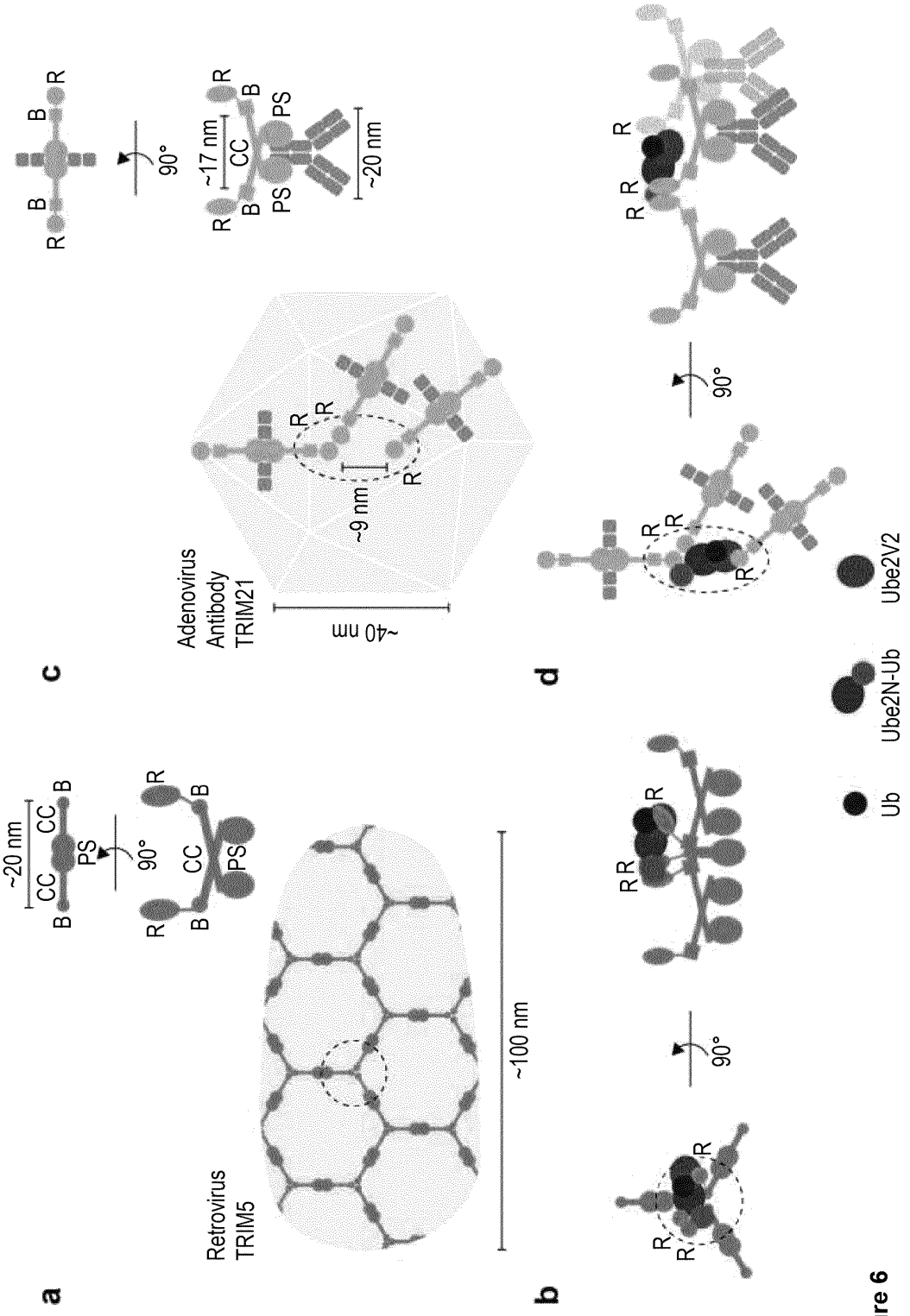

a
~20 nm
CC
CC
B
B
PS
90°
R
B
CC
PS
Retrovirus
TRIM5
~100 nm
b
R
R
90°
c
B
R
90°
~17 nm
CC
PS
~20 nm
Adenovirus
Antibody
TRIM21
~40 nm
~9 nm
d
R
90°
Ub
Ube2N-Ub
Ube2V2

Figure 6

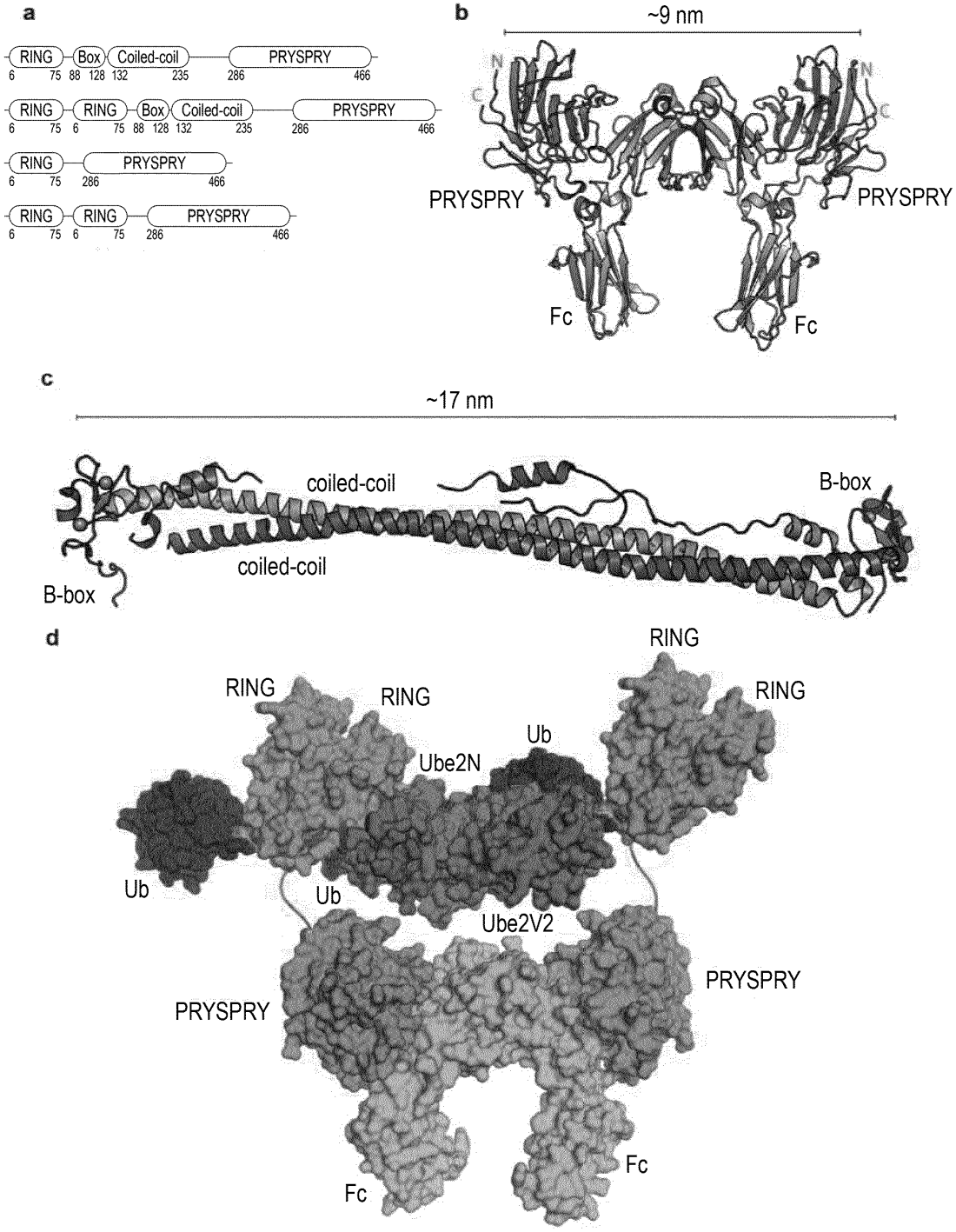

a
RING
Box
Coiled-coil
PRYSPRY
6 75 88 128 132 235 286 466
RING
RING
Box
Coiled-coil
PRYSPRY
6 75 6 75 88 128 132 235 286 466
RING
PRYSPRY
6 75 286 466
RING
RING
PRYSPRY
6 75 6 75 286 466
b
~9 nm
N
C
PRYSPRY
PRYSPRY
Fc
Fc
c
~17 nm
coiled-coil
B-box
coiled-coil
B-box
d
RING
RING
RING
RING
Ub
Ube2N
Ub
Ub
Ube2V2
PRYSPRY
PRYSPRY
Fc
Fc

Figure 7

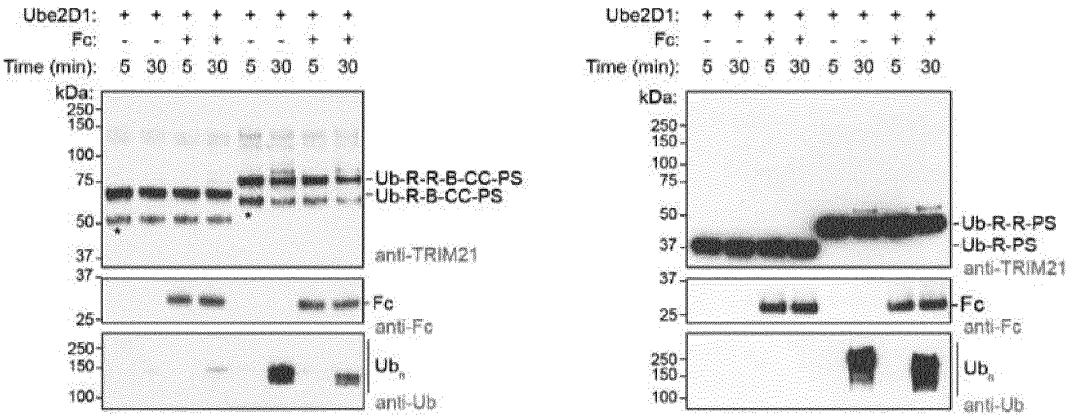


Figure 8

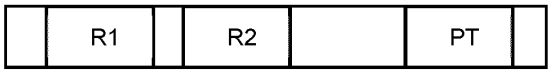


Figure 9

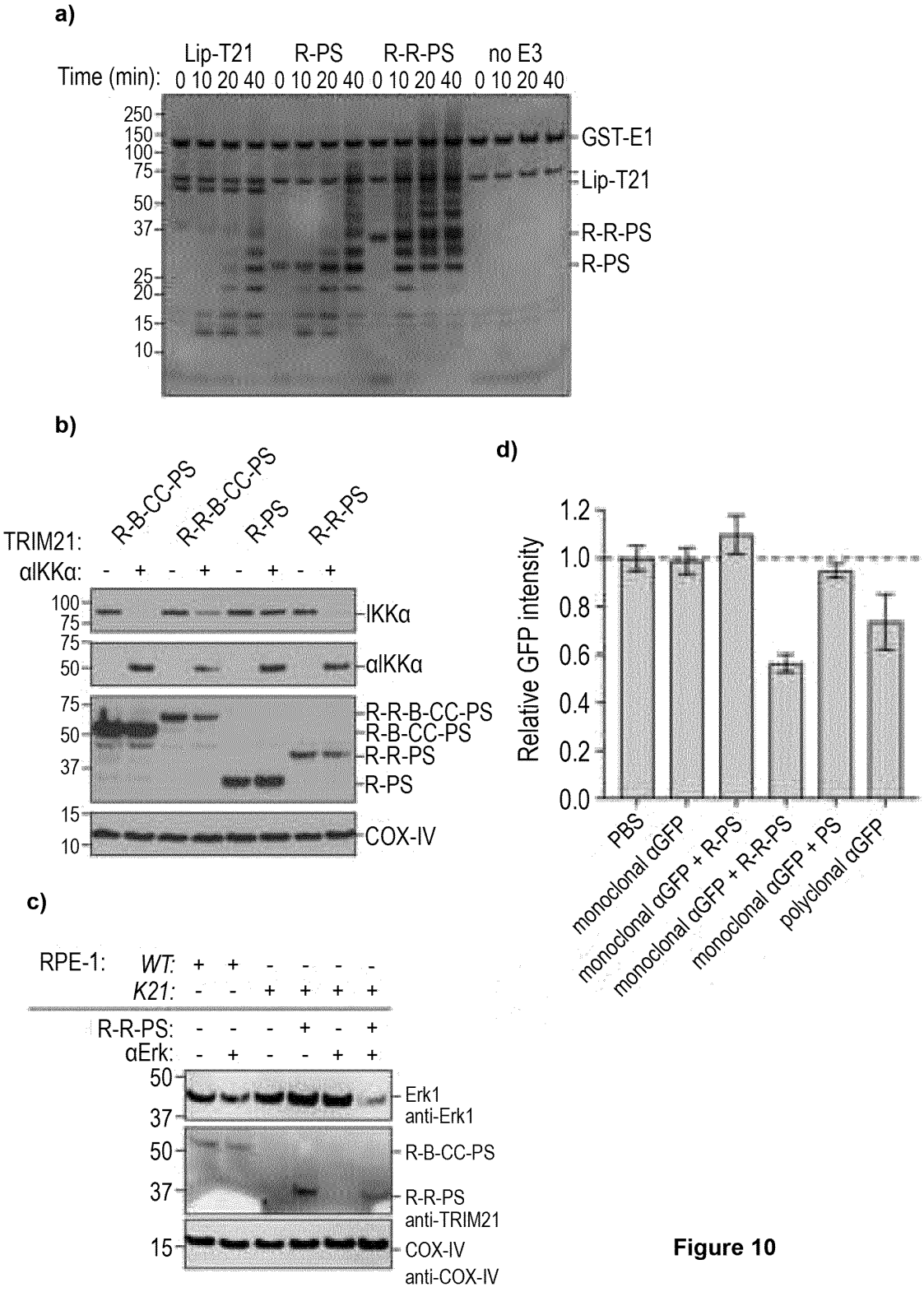
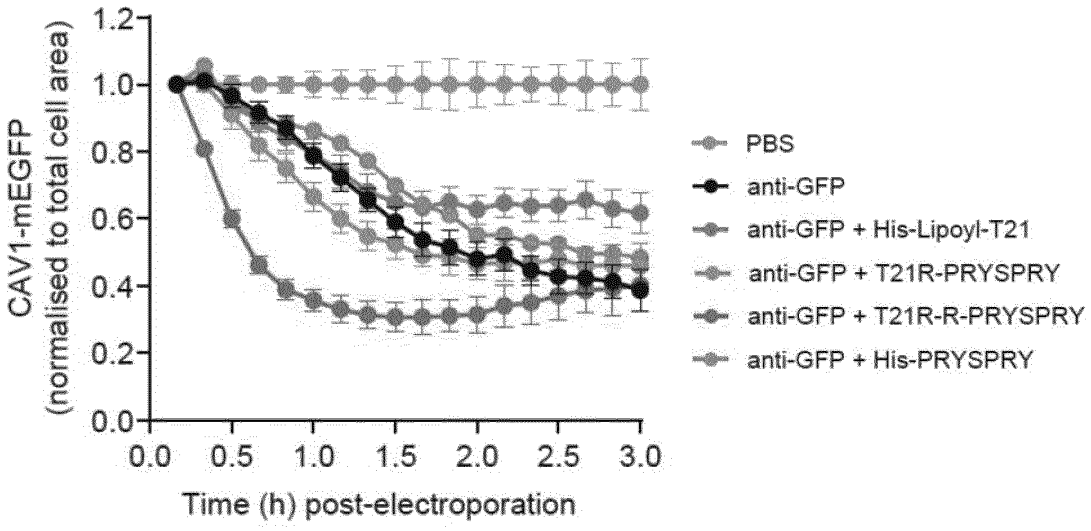
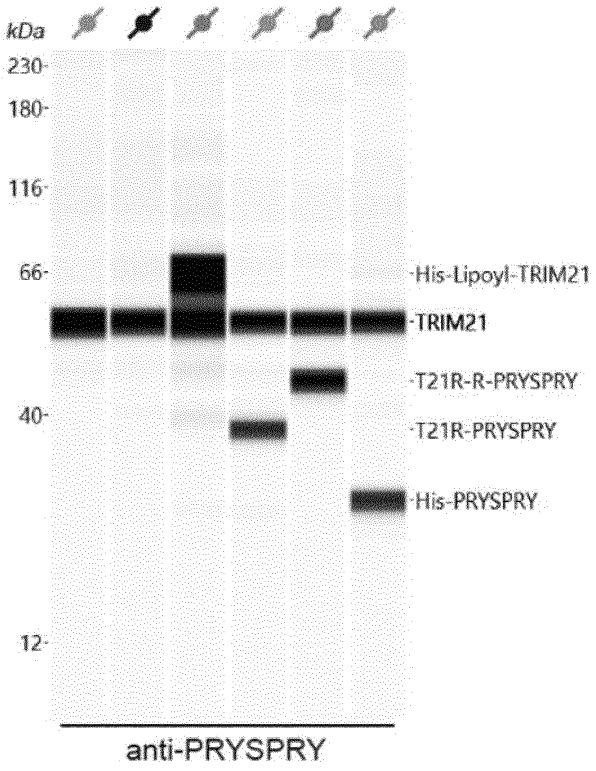

a)
Lip-T21 R-PS R-R-PS no E3
Time (min): 0 10 20 40 0 10 20 40 0 10 20 40 0 10 20 40
250
150
100
75
50
37
25
20
15
10
GST-E1
Lip-T21
R-R-PS
R-PS
b)
TRIM21: R-B-CC-PS R-R-B-CC-PS R-PS R-R-PS
αIKKα: - + - + - + - +
100
75
IKKα
75
50
αIKKα
75
50
37
R-R-B-CC-PS
R-B-CC-PS
R-R-PS
R-PS
15
10
COX-IV
c)
RPE-1: WT: + + - - - -
K21: - - + + + +
R-R-PS: - - - + - +
αErk: - + - - + +
50
37
Erk1
anti-Erk1
50
37
R-B-CC-PS
R-R-PS
anti-TRIM21
15
COX-IV
anti-COX-IV
d)
Relative GFP intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PBS
monoclonal αGFP
monoclonal αGFP + R-PS
monoclonal αGFP + R-R-PS
monoclonal αGFP + PS
polyclonal αGFP

Figure 10

a)



b)



c)

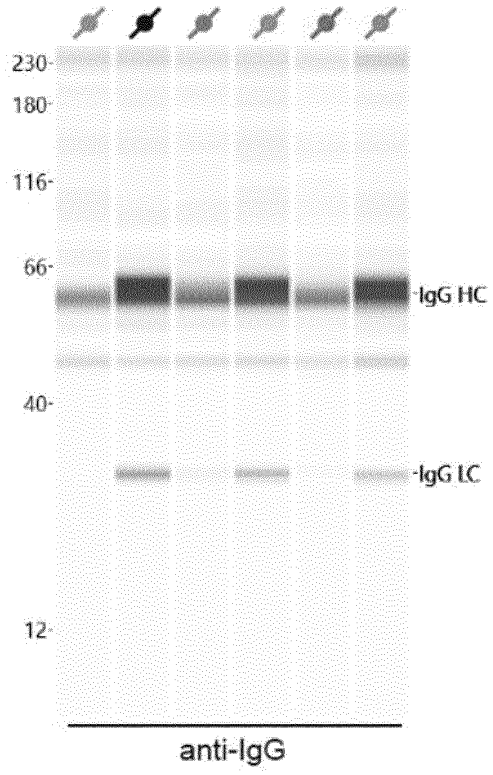


FIGURE 11

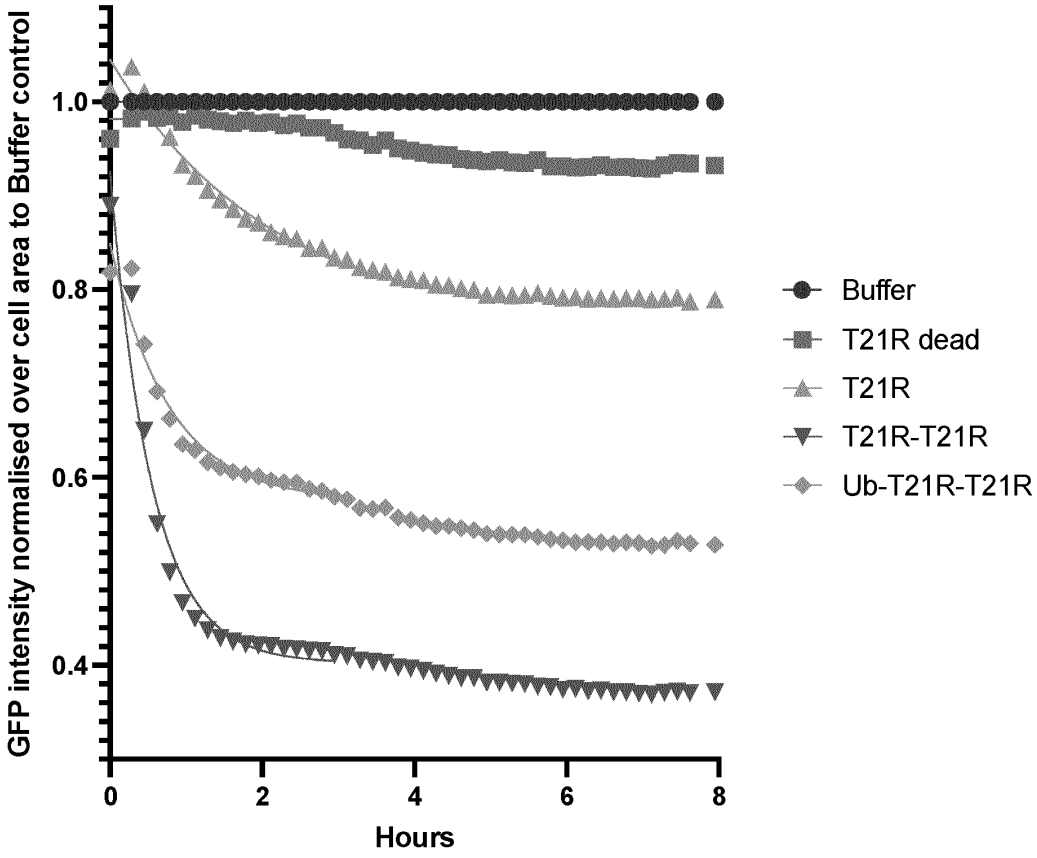

GFP intensity normalised over cell area to Buffer control
1.0
0.8
0.6
0.4
0
2
4
6
8
Hours
Buffer
T21R dead
T21R
T21R-T21R
Ub-T21R-T21R

FIGURE 12