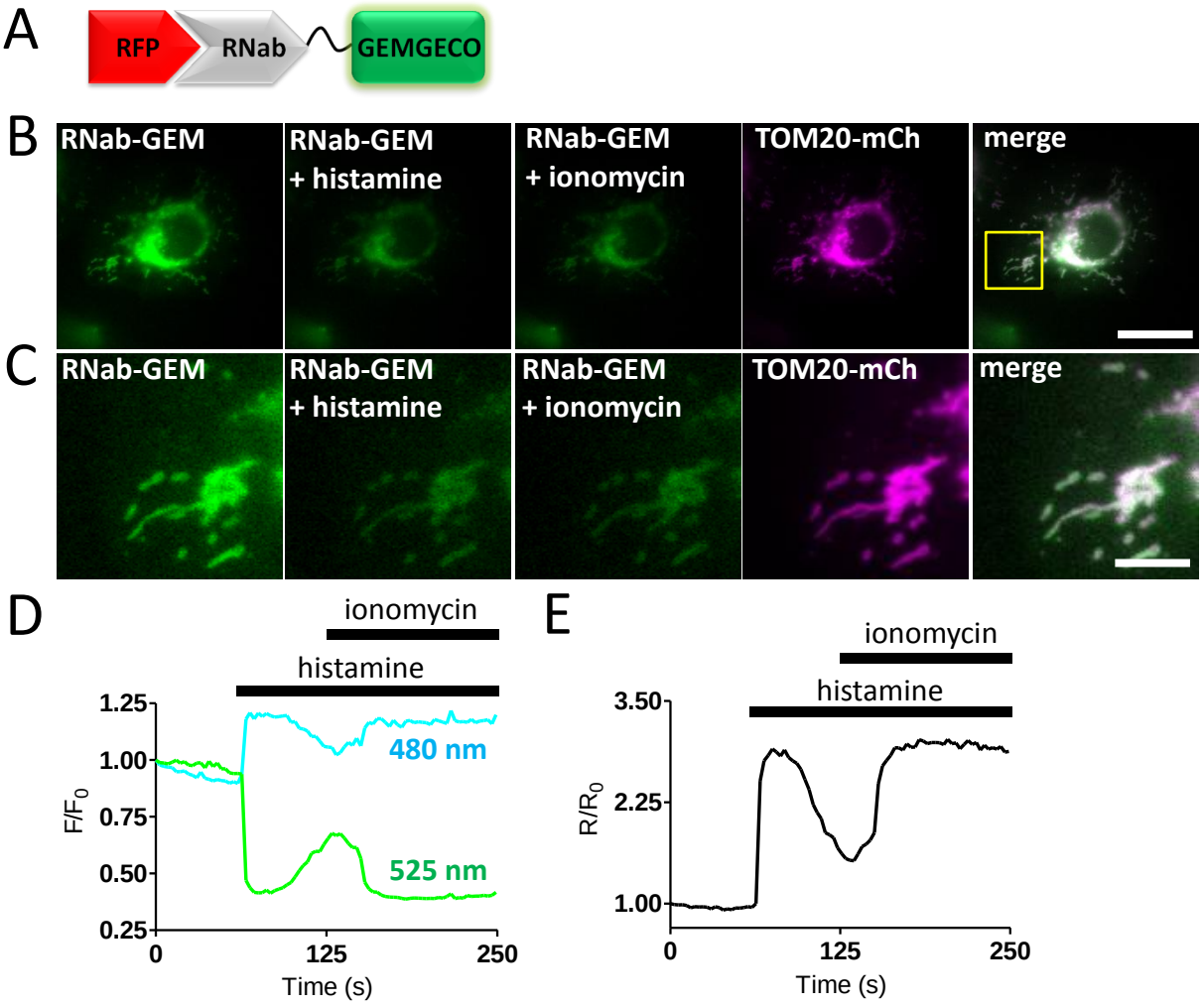
A
RFP
RNab
GEMGECO
B
RNab-GEM
RNab-GEM
+ histamine
RNab-GEM
+ ionomycin
TOM20-mCh
merge
C
RNab-GEM
RNab-GEM
+ histamine
RNab-GEM
+ ionomycin
TOM20-mCh
merge
D
ionomycin
histamine
480 nm
525 nm
F/F0
1.25
1.00
0.75
0.50
0.25
0
125
250
Time (s)
E
ionomycin
histamine
R/R0
3.50
2.25
1.00
0
125
250
Time (s)

Figure S1

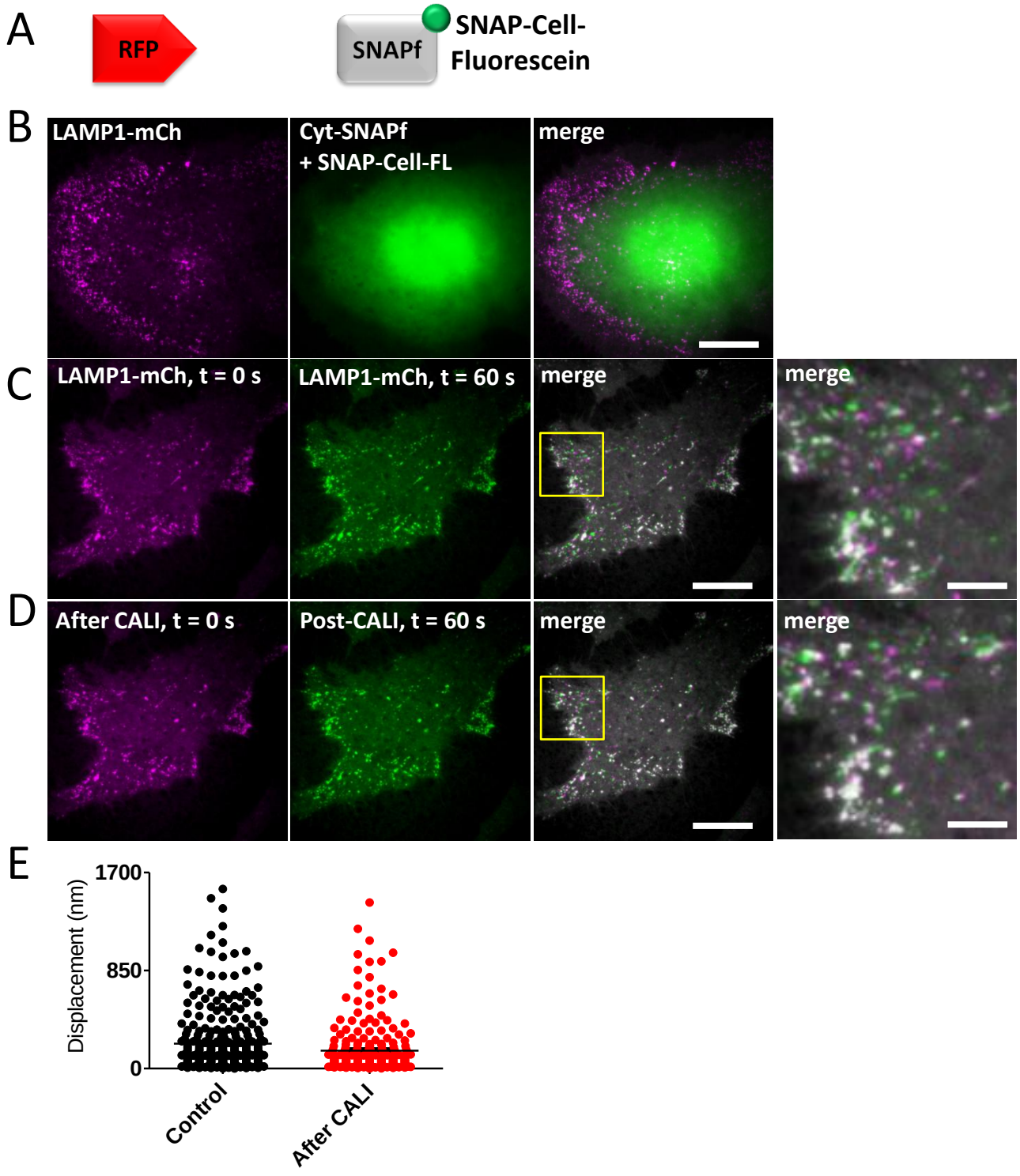
A
RFP
SNAPf
SNAP-Cell-
Fluorescein
B
LAMP1-mCh
Cyt-SNAPf
+ SNAP-Cell-FL
merge
C
LAMP1-mCh, t = 0 s
LAMP1-mCh, t = 60 s
merge
merge
D
After CALI, t = 0 s
Post-CALI, t = 60 s
merge
merge
E
Displacement (nm)
1700
850
0
Control
After CALI

Figure S2

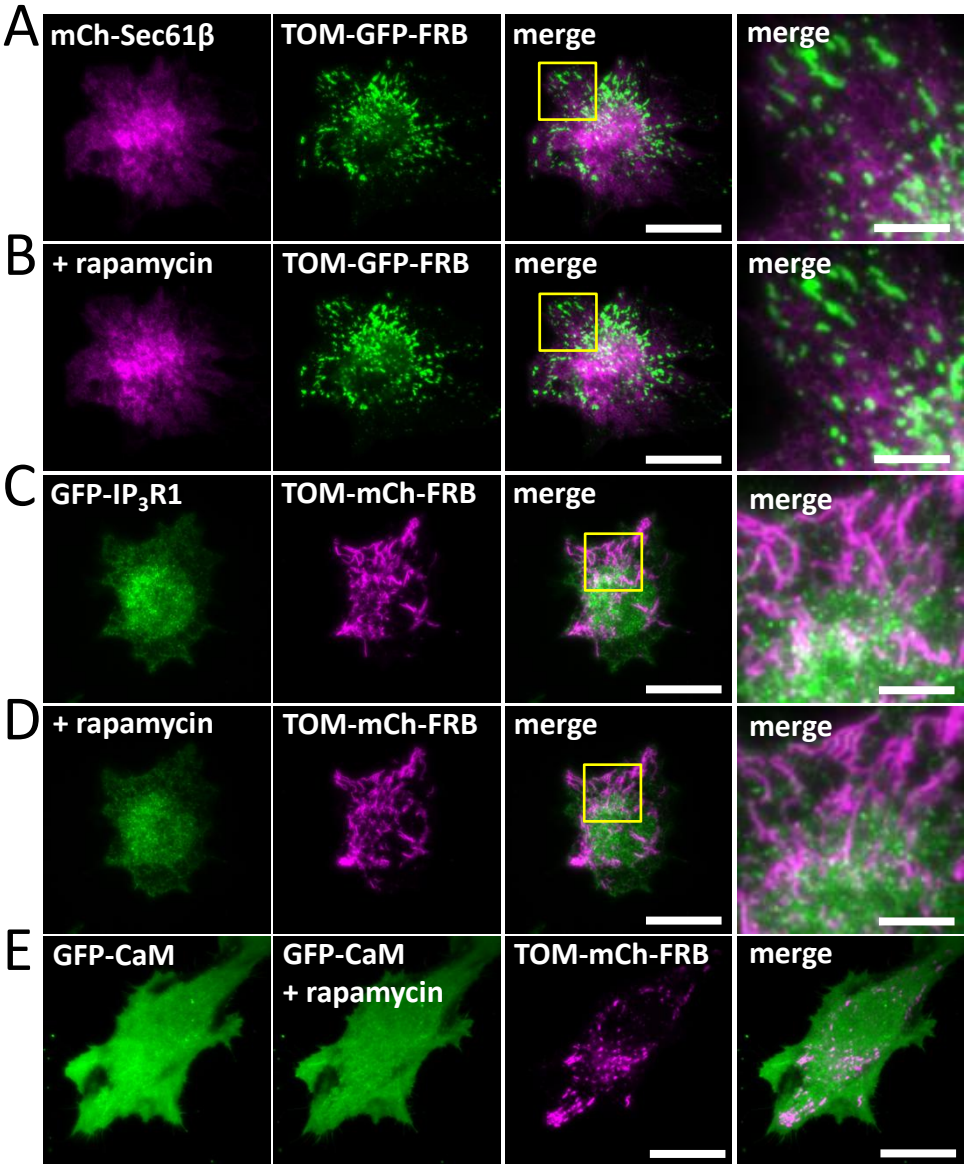
A
mCh-Sec61β
TOM-GFP-FRB
merge
merge
B
+ rapamycin
TOM-GFP-FRB
merge
merge
C
GFP-IP3R1
TOM-mCh-FRB
merge
merge
D
+ rapamycin
TOM-mCh-FRB
merge
merge
E
GFP-CaM
GFP-CaM
+ rapamycin
TOM-mCh-FRB
merge

Figure S3

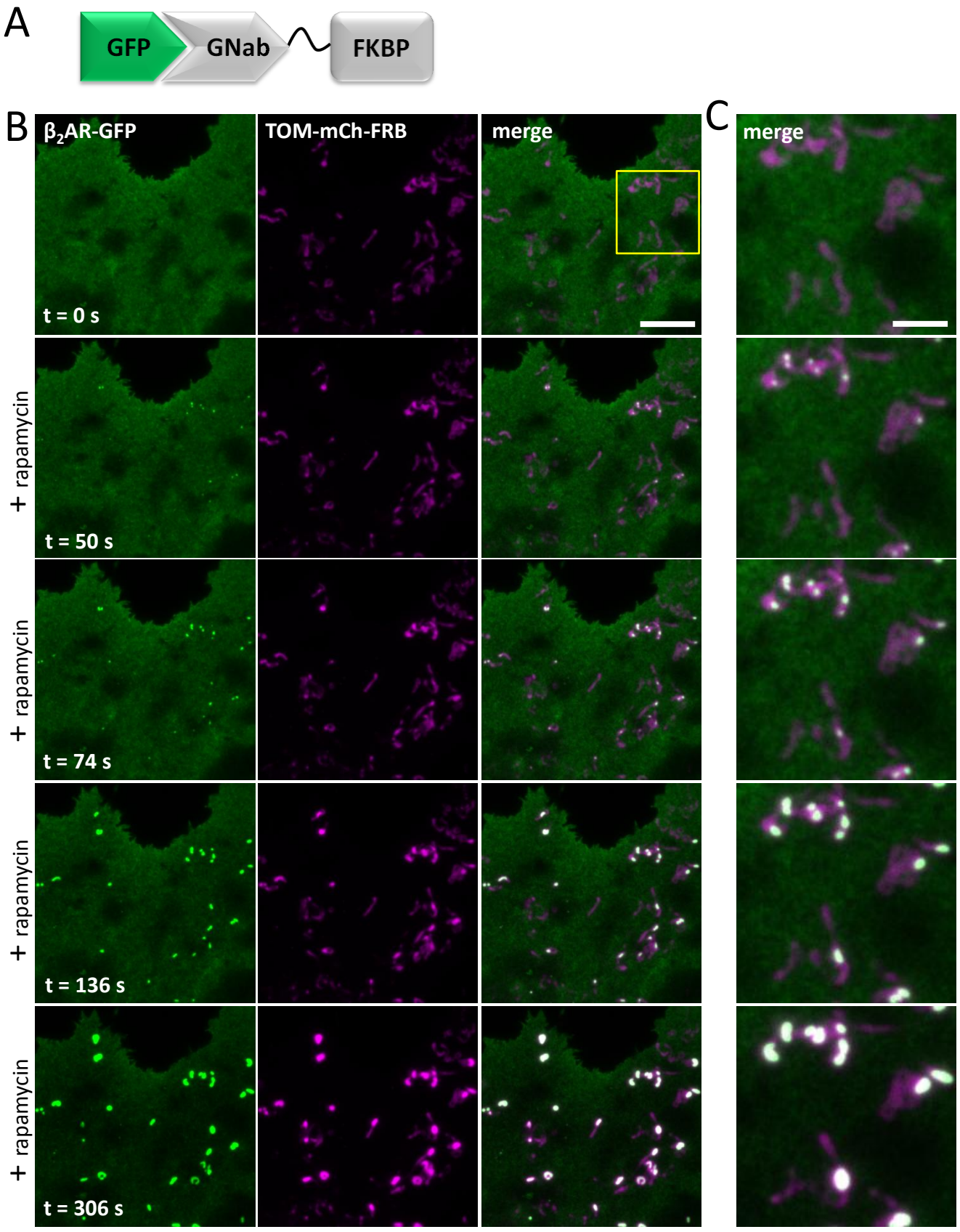
A
GFP
GNab
FKBP
B
β2AR-GFP
TOM-mCh-FRB
merge
C
merge
t = 0 s
+ rapamycin
t = 50 s
+ rapamycin
t = 74 s
+ rapamycin
t = 136 s
+ rapamycin
t = 306 s

Figure S4

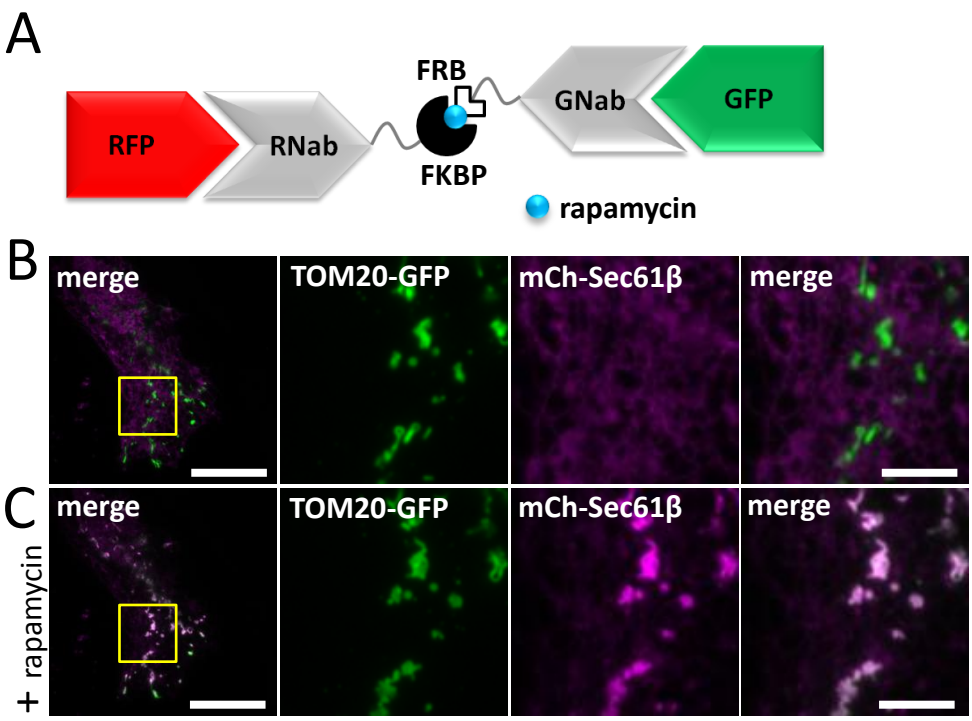
A
RFP
RNab
FRB
FKBP
GNab
GFP
rapamycin
B
merge
TOM20-GFP
mCh-Sec61β
merge
C
+ rapamycin
merge
TOM20-GFP
mCh-Sec61β
merge

Figure S5

Figure S6. DNA sequences encoding the nanobodies used.

ATG – start codon of region encoding nanobody

NANOBODY – region encoding nanobody

LINKER – region encoding flexible linker between nanobody and functional module

TXX – stop codon

GNab

AAGCTTGCCACCATGGCTCAGGTGCAGCTGGTGGAATCTGGCGGCAGACTGGTGCAGGC
CGGCGATAGCCTGAGACTGTCTTGTGCCGCCAGCGGCAGAACCTTCAGCACATCTGCCAT
GGCCTGGTTCAGACAGGCCCCTGGCCGCGAGAGGGAATTTGTGGCCGCCATCACATGGA
CCGTGGGCAACACCATCCTGGGCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGAC
AGAGCCAAGAACACCGTGGACCTCCAGATGGACAACCTGGAACCCGAGGACACCGCCGT
GTACTACTGCTCCGCCAGATCCAGAGGCTACGTGCTGTCCGTGCTGCGGAGCGTGGACAG
CTACGATTATTGGGGCCAGGGCACCCAAGTGACCGTGTCTGGCGGCGGAGGAAGCGGAG
GCGGAGGATCTGGGGGAGGCGGCAGTGGCGGAGGGGGATCTGGATCC

RNab

AAGCTTGCCACCATGGCTCAGGTGCAGCTGGTGGAAAGCGGCGGCTCTCTGGTGCAGCC
TGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGATTTGCCGAGAGCAGCAGCA
TGGGCTGGTTCAGACAGGCCCCTGGCAAAGAACGCGAGTTCGTGGCCGCCATCTCTTGG
AGCGGCGGAGCCACCAATTACGCCGATAGCGCCAAGGGCCGGTTCACCCTGAGCCGGGA
CAACACCAAGAACACCGTGTACCTGCAGATGAACAGCCTGAAGCCCGACGACACCGCCG
TGTACTACTGCGCCGCCAACCTGGGCAACTACATCAGCAGCAACCAGCGGCTGTACGGC
TACTGGGGCCAGGGAACACAAGTGACCGTGTCCAGCCCTTTCACAGGCGGCGGAGGATC
TGGCGGAGGCGGATCTGGGGGCGGAGGCTCTGGATCC

GNab-RNab

AAGCTTGCCACCATGGCTCAGGTGCAGCTGGTTGAATCTGGCGGCAGACTGGTTCAGGCC
GGCGATTCTCTGAGACTGTCTTGTGCCGCCAGCGGCAGAACCTTTAGCACATCTGCCATG
GCCTGGTTCAGACAGGCCCCTGGAAGAGAACGGGAATTTGTGGCCGCCATCACCTGGAC
CGTGGGCAATACCATCCTGGGCGATAGCGTGAAGGGCAGATTCACCATCAGCCGGGACA
GAGCCAAGAACACCGTGGACCTCCAGATGGACAACCTGGAACCTGAGGACACCGCCGTG
TACTACTGCTCCGCTAGAAGCAGAGGCTACGTGCTGTCCGTGCTGAGAAGCGTGGACAG
CTACGATTATTGGGGCCAGGGCACCCAAGTGACCGTTTCTGGTGGCGGAGGATCTGGCG
GAGGTGGAAGCGGCGGAGGCGGTAGCGGAGGTGGTGGATCTGGATCCATGGCCCAGGTC
CAGCTCGTGGAAAGTGGCGGATCTCTGGTTCAACCTGGCGGAAGCCTGAGACTGAGCTG
TGCCGCTTCTGGCAGATTTGCCGAGAGCAGCAGCATGGGCTGGTTTAGGCAAGCCCCAG
GCAAAGAGAGAGAGTTCGTCGCCGCCATCTCTTGGAGTGGCGGAGCCACCAATTACGCC
GATTCTGCCAAAGGCCGGTTCACCCTGAGCAGAGACAACACAAAGAATACGGTGTATCT
CCAGATGAACTCCCTGAAGCCAGACGATACAGCCGTGTATTATTGCGCCGCCAACCTGG
GCAACTACATCAGCAGCAACCAGCGGCTGTACGGCTACTGGGGACAGGGAACACAAGTC
ACAGTGTCTAGCCCCTTCACCTGACTGCAGATATC