Supplemental Figure Legends

Supplemental Figure S1. Effect of H89 on the distribution of COPI and COPII. COS-7 cells were incubated with H89 at the indicated concentrations for 15 min, and double stained for Bap31 and Sec31A (left three columns) or β-COP (right three columns). Scale bar, 10 μm

Supplemental Figure S2. Bap31, unlike conventional cargos, does not accumulate at ER exit sites in Noc-treated cells. COS-7 cells were incubated with dimethyl sulfoxide or 5 μg/ml Noc for 3 h and then plus 50 μM H89 for 15 min. They were double stained for Bap31 and SERCA2 (top two rows), Sec31A (third row), or β-COP (fourth row), or GM130 and Sec31p (bottom row). The boxed areas are enlarged in the insets. Scale bars, 5 μm.

Supplemental Figure S3. Time courses of GM130 assembly and Bap31 cycling after BFA washout. COS-7 cells were incubated with 10 μM BFA for 30 min, and then washed to remove BFA. Change in the localization of GM130 (left column) and Bap31 (middle column). Scale bar, 10 μm

Supplemental Figure S4. Live cell imaging of the movement of Bap31-mRFP and NAGFP during recovery from BFA treatment. Tet-on HeLa cells expressing both Bap31-mRFP and

NAGFP were monitored after BFA washout. Arrows indicate the movement of an NAGFP positive dot. Arrowheads denote the accumulation of Bap31 at the juxtanuclear region. Scale bars, 10 μm.

Supplemental Figure S5. Stably expressed Bap31-mRFP behaves similarly to endogenous Bap31. (A) Change in the localization of endogenous Bap31 and Bap31-mRFP during recovery from BFA treatment. Parental Tet-on HeLa cells (left column) and those stably expressing Bap31-mRFP (right column). Scale bar, 10 μm. (B) Quantitation of cells with the juxtanuclear accumulation of Bap31-mRFP and endogenous Bap31.

Supplemental Figure S6. Behaviors of ERGIC-53 and Bap31 after BFA washout. HeLa cells were incubated with 10 μM BFA for 30 min, and then washed to remove BFA. At the indicated times, the cells were double stained for ERGIC-53 (left column) and Bap31 (middle column). Scale bars, 10 μm

Supplemental Figure 7. Localization of chimeras between Bap31 and Bap29. At 3 h after transfection of the plasmids for the indicated chimeras, Tet-on HeLa cells were incubated with the 1 μg/ml doxycycline for 40 h to induce protein expression. The cells were analyzed immediately

(Untreated) or subjected to BFA/washout treatment. Scale bar, 10 μm.

Supplemental Figure S8. Distribution of 29(65)31-mRFP at 15ºC. Tet-on HeLa cells expressing 29(65)31-mRFP were incubated at 15ºC for 2 h, and then stained ERGIC-53. Scale bar, 10 μm

Figure S1.

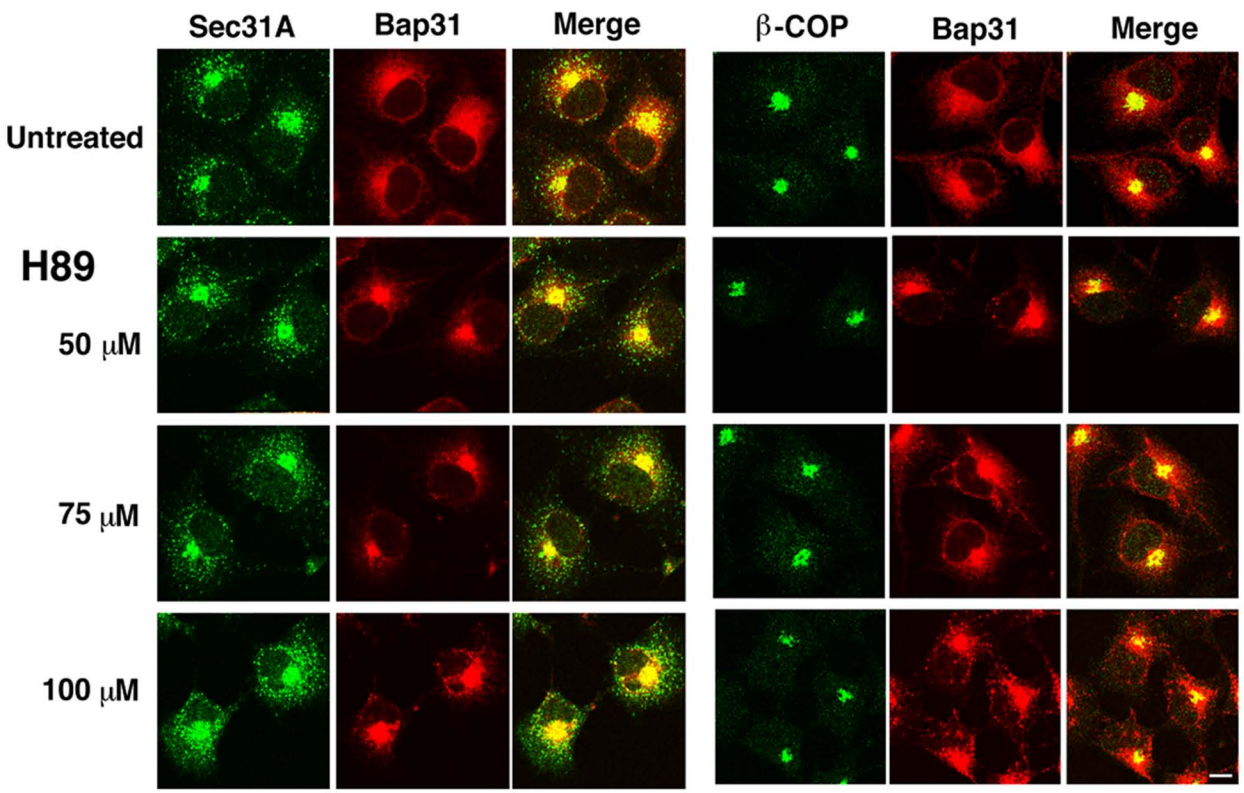
Sec31A
Bap31
Merge
β-COP
Bap31
Merge
Untreated
H89
50 μM
75 μM
100 μM

Figure S2.

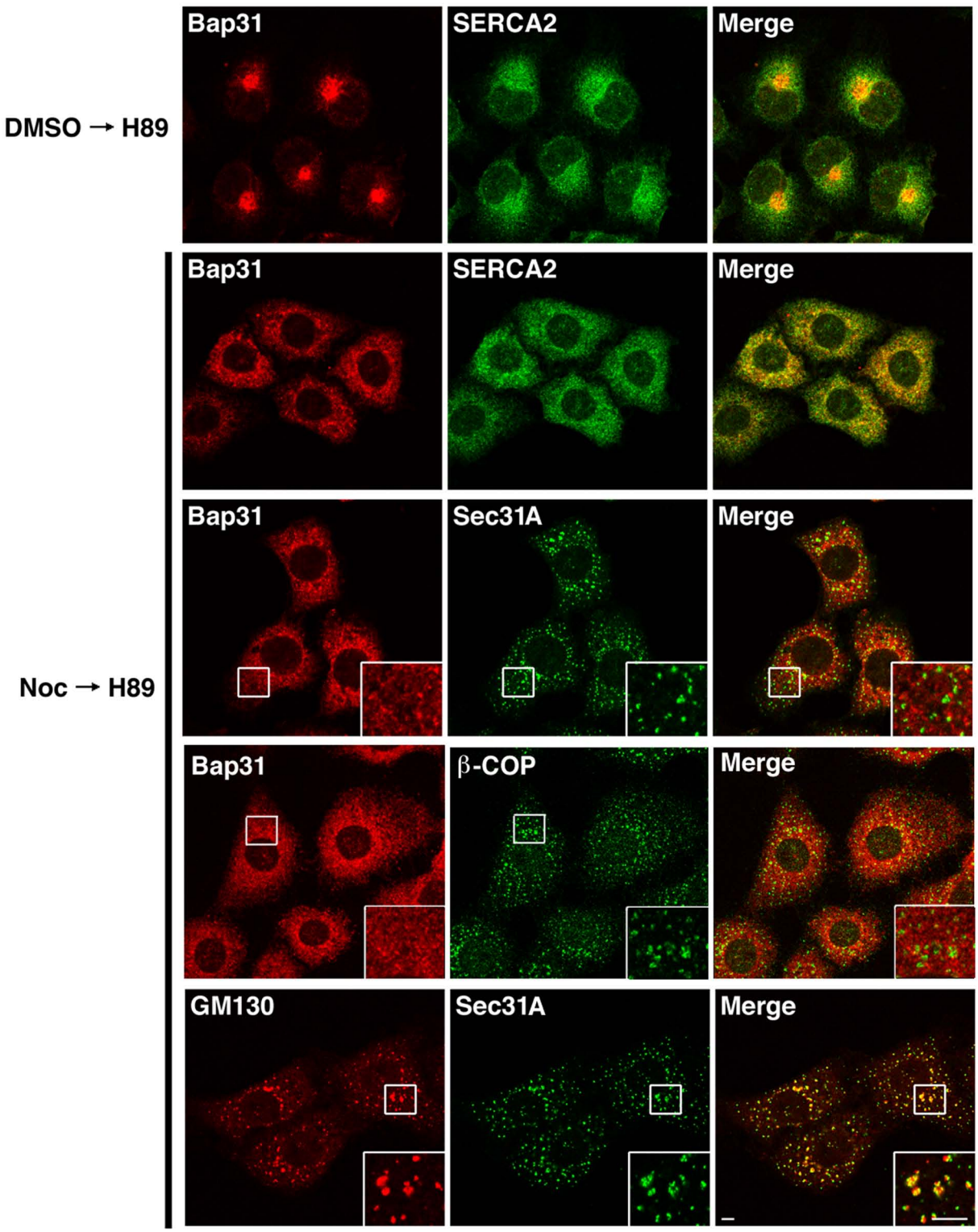
DMSO → H89
Bap31
SERCA2
Merge
Noc → H89
Bap31
SERCA2
Merge
Bap31
Sec31A
Merge
Bap31
β-COP
Merge
GM130
Sec31A
Merge

Figure S3.

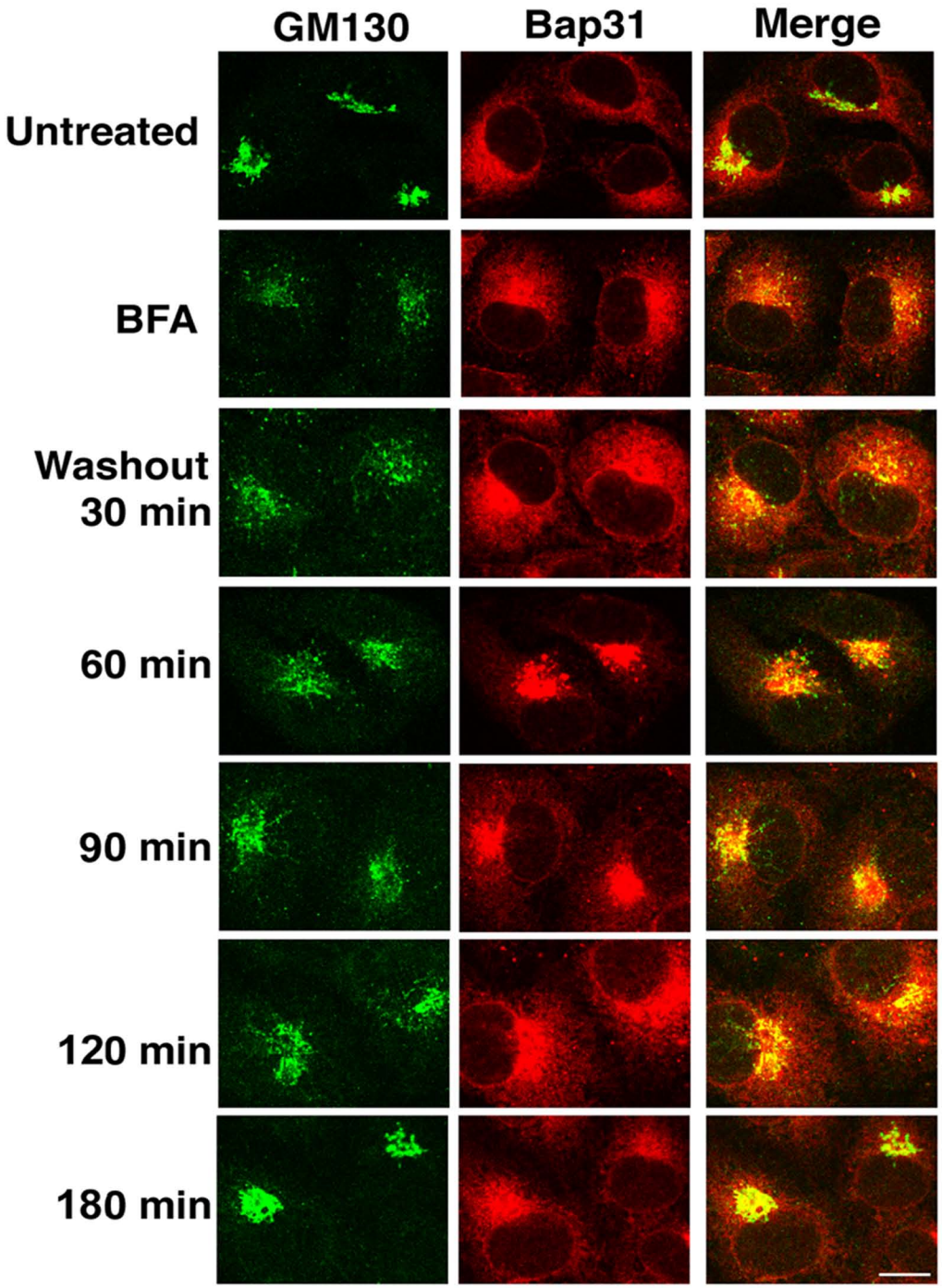
GM130
Bap31
Merge
Untreated
BFA
Washout
30 min
60 min
90 min
120 min
180 min

Figure S4.

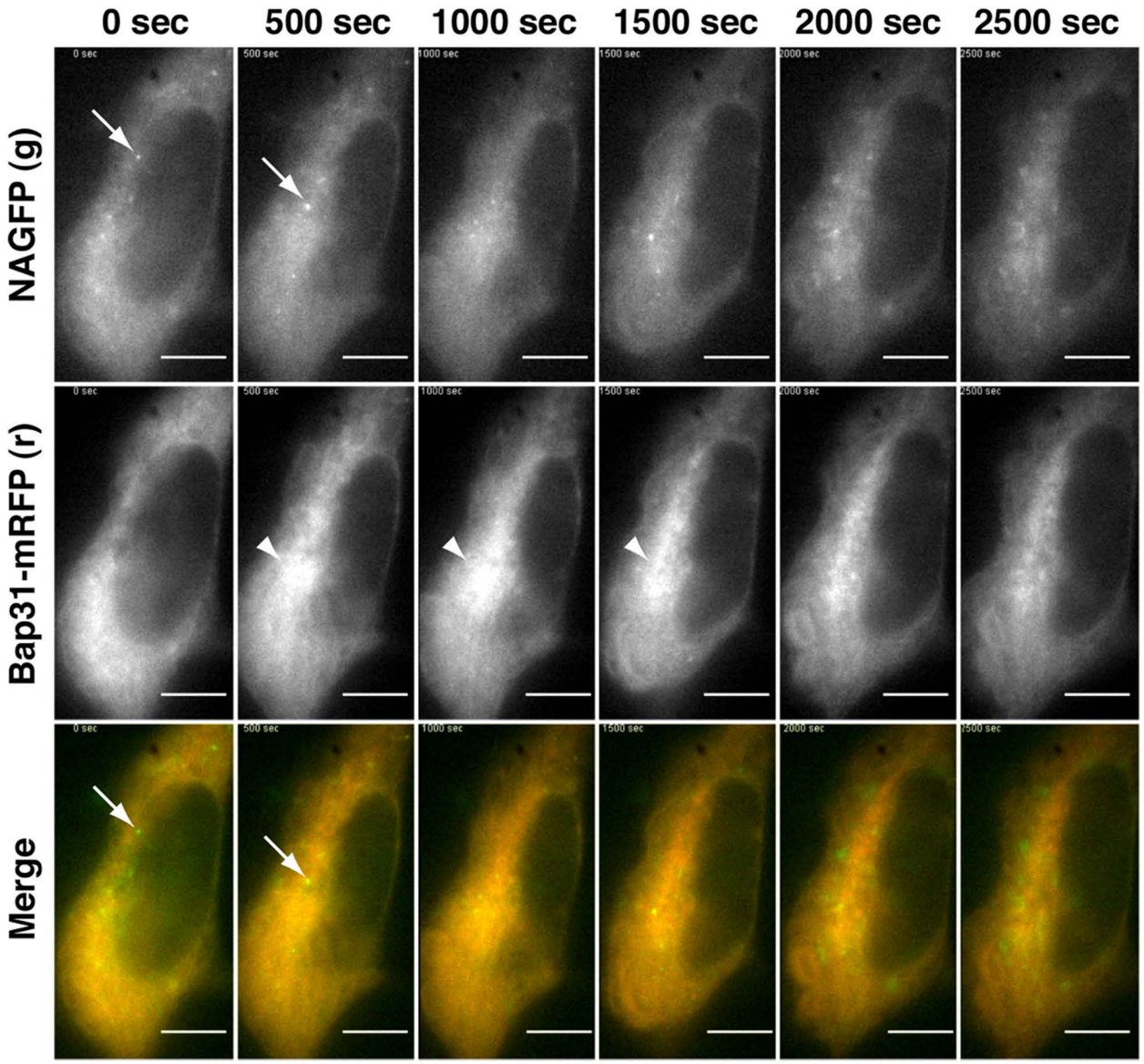
0 sec
500 sec
1000 sec
1500 sec
2000 sec
2500 sec
NAGFP (g)
Bap31-mRFP (r)
Merge

Figure S5.

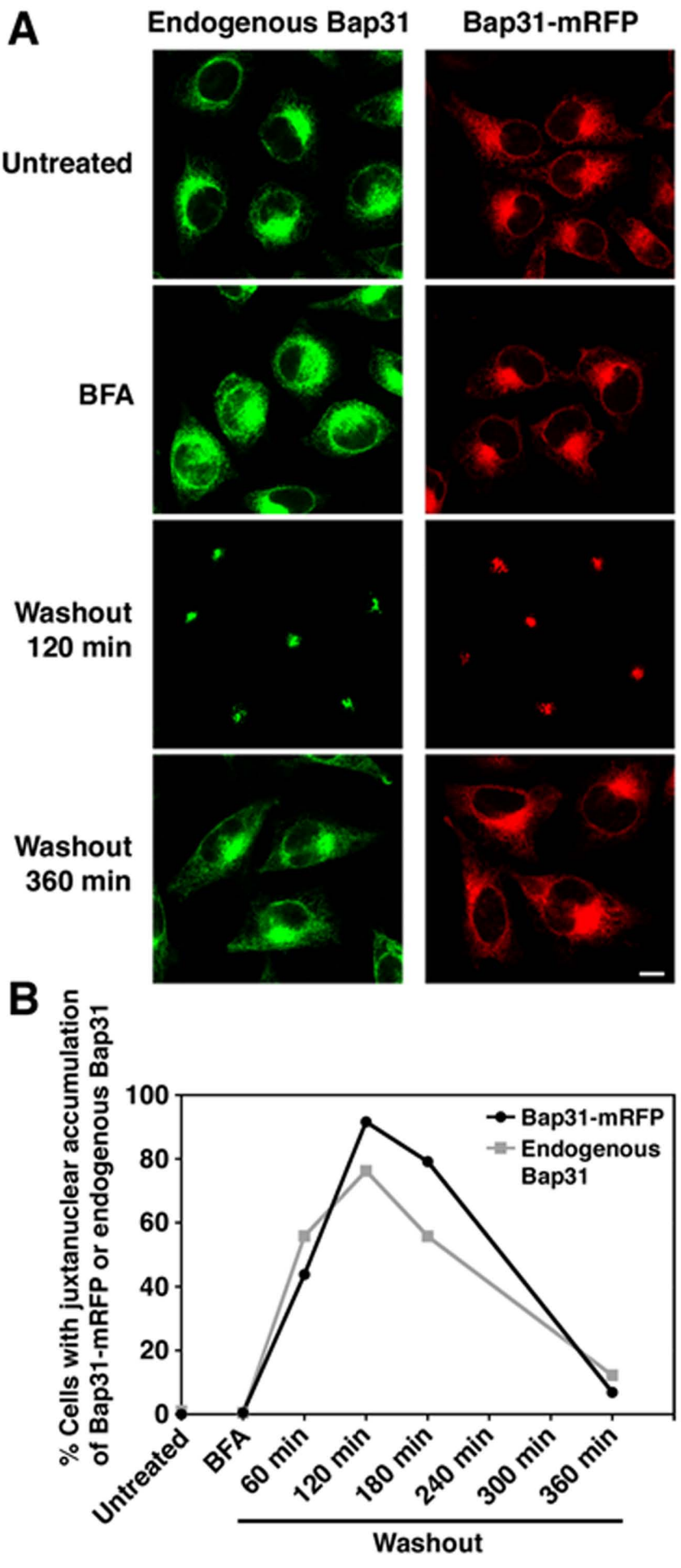
A
Endogenous Bap31
Bap31-mRFP
Untreated
BFA
Washout
120 min
Washout
360 min
B
% Cells with juxtanuclear accumulation of Bap31-mRFP or endogenous Bap31
100
80
60
40
20
0
Bap31-mRFP
Endogenous Bap31
Untreated
BFA
60 min
120 min
180 min
240 min
300 min
360 min
Washout

Figure S6.

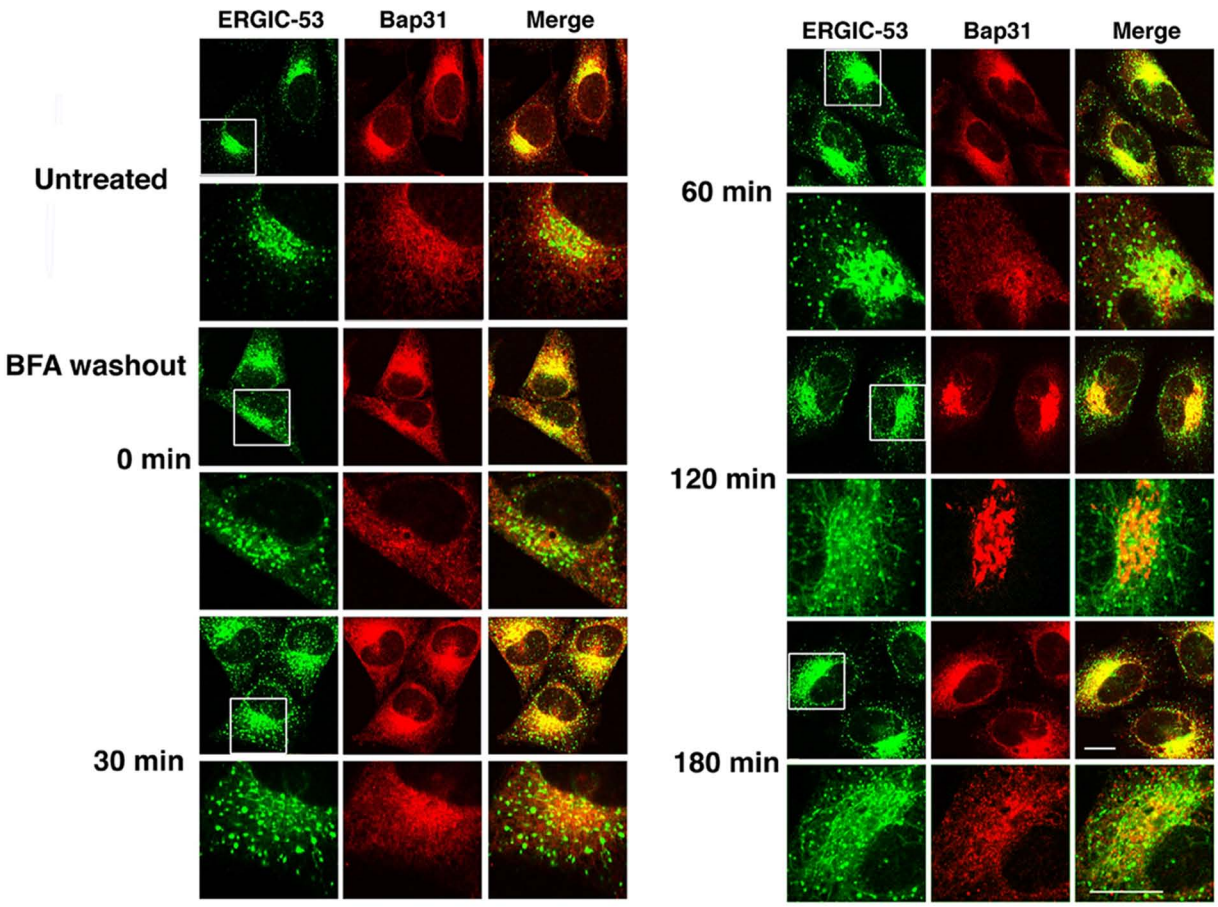
ERGIC-53
Bap31
Merge
Untreated
BFA washout
0 min
30 min
60 min
120 min
180 min

Figure S7.

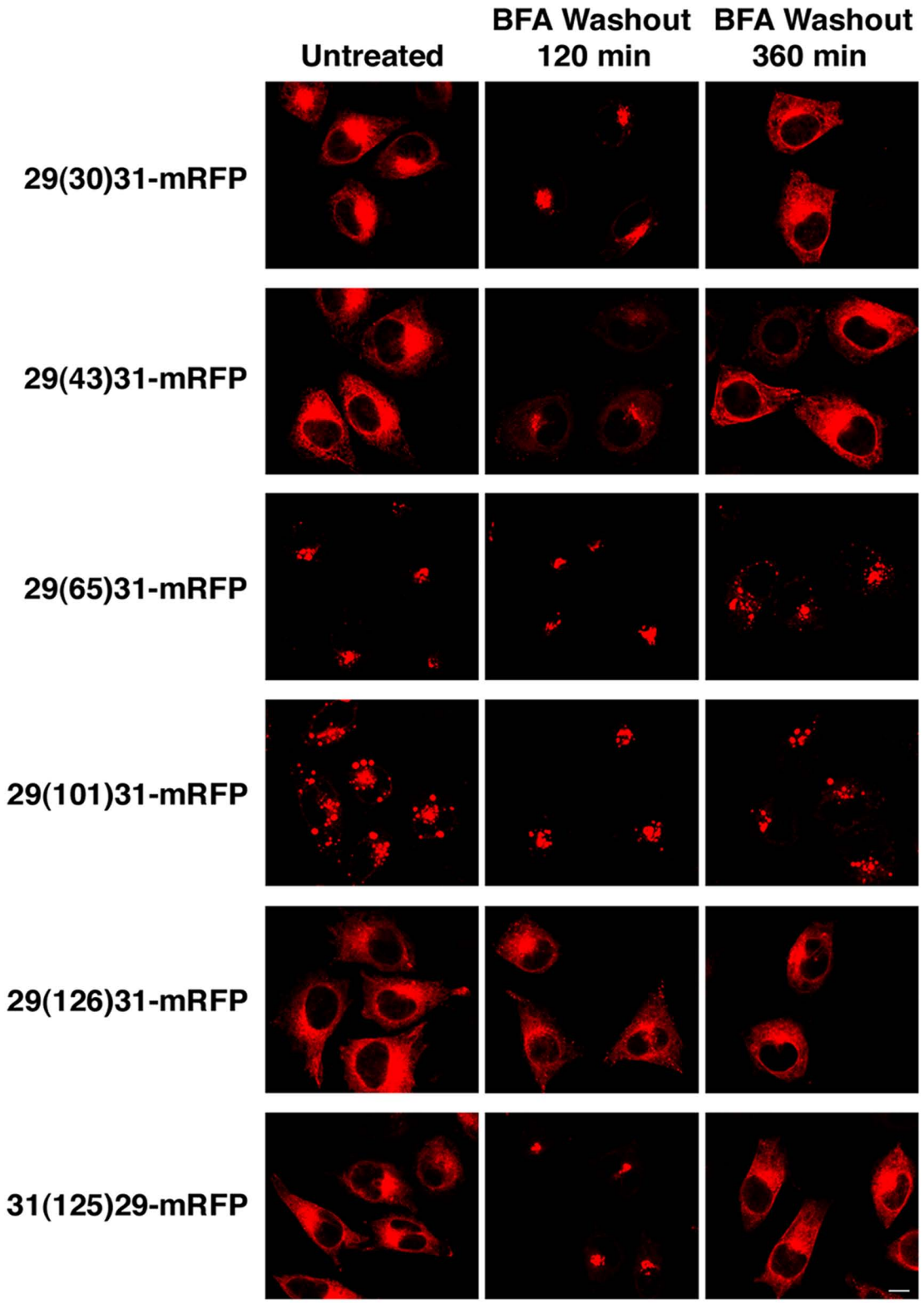
Untreated
BFA Washout 120 min
BFA Washout 360 min
29(30)31-mRFP
29(43)31-mRFP
29(65)31-mRFP
29(101)31-mRFP
29(126)31-mRFP
31(125)29-mRFP

Figure S8.