Supplementary Figures

Figure S1. ***Investigation of the possible rearrangement of the endomembrane targeted constructs upon PM PPIns depletion***
To determine the possible movement of endomembrane targeted Luciferase constructs upon PM PPIns depletion, fluorescently (Cerulean) tagged versions were transiently expressed in HEK 293T cells co-expressing both proteins of the depletion system (PM-FRB and mRFP-FKBP-PJ, not visible). Upper pictures show the control period, whereas the lower images were collected 5 min after rapamycin (300 nM) treatment. Cells are representatives of at least ten measurements carried out in three independent experiments.

Figure S2. ***Investigation of the direct effect of deltarasine and PI4KA inhibitor A1 on the Venus-K-Ras-CAAX Golgi localization without a concomitant PM PPIns depletion***
(A) Effect of 10 μM deltarasine on the Venus-K-Ras-CAAX Golgi localization, determined by interaction measurements between Venus-K-Ras-CAAX and Golgi targeted Luciferase (Golgi-Luc) using BRET technique. Difference of the BRET ratios was calculated between deltarasine (10 μM) and vehicle (DMSO) treated cells. Data are means ± SEM of three independent experiments. Statistical analysis were performed using *t*-test (*$p < 0.05$.)
(B) Effect of specific PI4KA inhibitor A1 compound (10 nM) on the Venus-K-Ras-CAAX Golgi localization, determined by interaction measurements between Venus-K-Ras-CAAX and Golgi targeted Luciferase (Golgi-Luc) using BRET technique. The measurements were carried out on transiently transfected HEK 293T cells expressing the indicated constructs for BRET measurement. Difference of the BRET ratios was calculated between A1 (10 nM) and vehicle (DMSO) treated cells. Data are means ± SEM of three independent experiments. Statistical analysis were performed using *t*-test (*$p < 0.05$).

Figure S3. ***Comparison of the Golgi translocation of the constitutively active, wild type and dominant negative full-length forms of fluorescently tagged K- and H-Ras constructs after PM PPIns depletion***
(A) Effect of the PM PPIns depletion on the K-Ras translocation to the Golgi, using constitutively activeG12V, wild type (WT) and dominant negative S17N forms of Venus tagged full-length Ras constructs and the same Golgi targeted Luciferase protein (Golgi-Luc), which were used in previous experiments. Difference of the BRET ratios was calculated between rapamycin (300 nM) and vehicle (DMSO) treated cells. Data are means ± SEM of three independent experiments. Statistical analysis were performed using *t*-test (**$p < 0.01$).
(B) Effect of the PM PPIns depletion on the H-Ras translocation to the Golgi, using constitutively activeG12V, wild type (WT) and dominant negative S17N forms of Venus tagged full-length Ras constructs and the same Golgi targeted Luciferase protein (Golgi-Luc), which were used in previous experiments. The measurements were carried out on transiently transfected HEK 293T cells expressing the required proteins of the depletion system (Lck_{1-10}-FRB-mRFP and mRFP-FKBP-PJ) and the indicated constructs for the BRET measurements. Difference of the BRET ratios was calculated between rapamycin (300 nM) and vehicle (DMSO) treated cells. Data are means ± SEM of three independent experiments. Statistical analysis were performed using *t*-test (ns, non-significant).

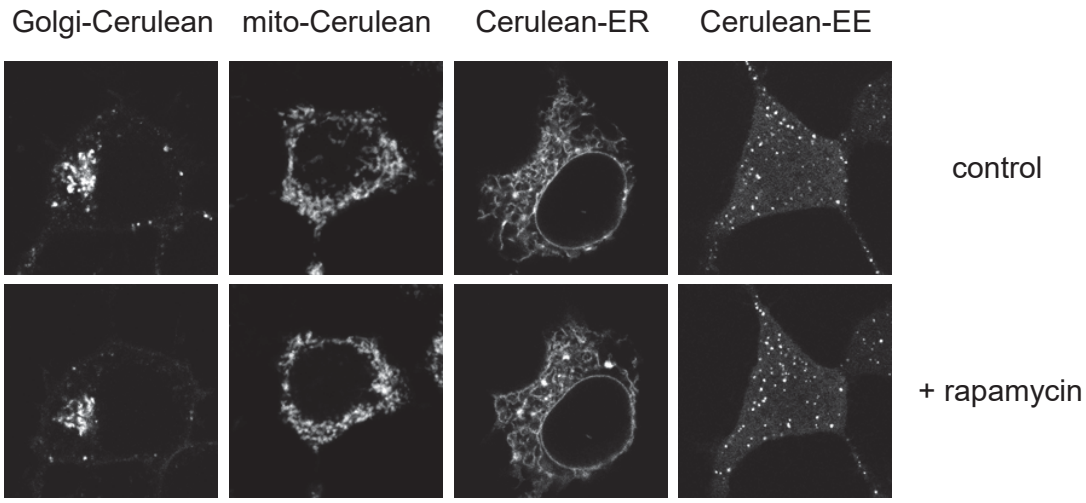
Golgi-Cerulean
mito-Cerulean
Cerulean-ER
Cerulean-EE
control
+ rapamycin

Figure S1

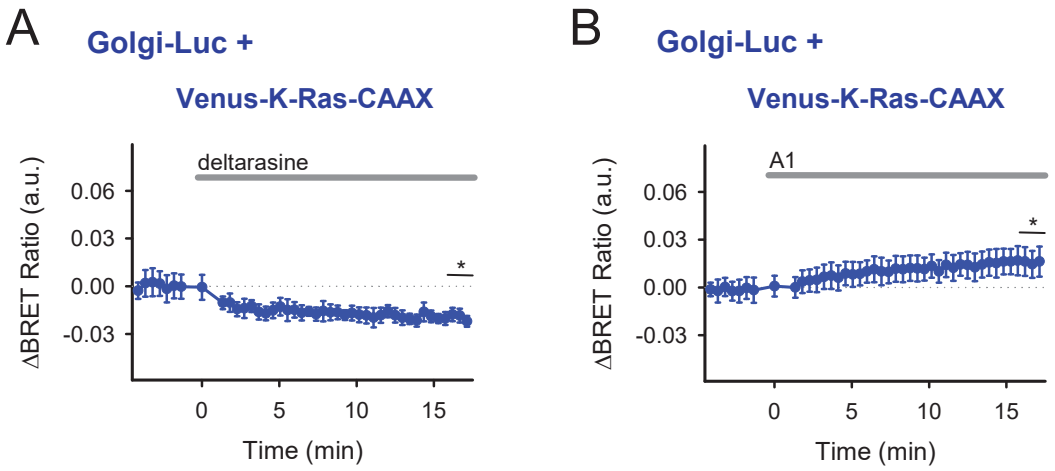
A
Golgi-Luc +
Venus-K-Ras-CAAX
deltarasine
ΔBRET Ratio (a.u.)
0.06
0.03
0.00
-0.03
*
0
5
10
15
Time (min)
B
Golgi-Luc +
Venus-K-Ras-CAAX
A1
ΔBRET Ratio (a.u.)
0.06
0.03
0.00
-0.03
*
0
5
10
15
Time (min)

Figure S2

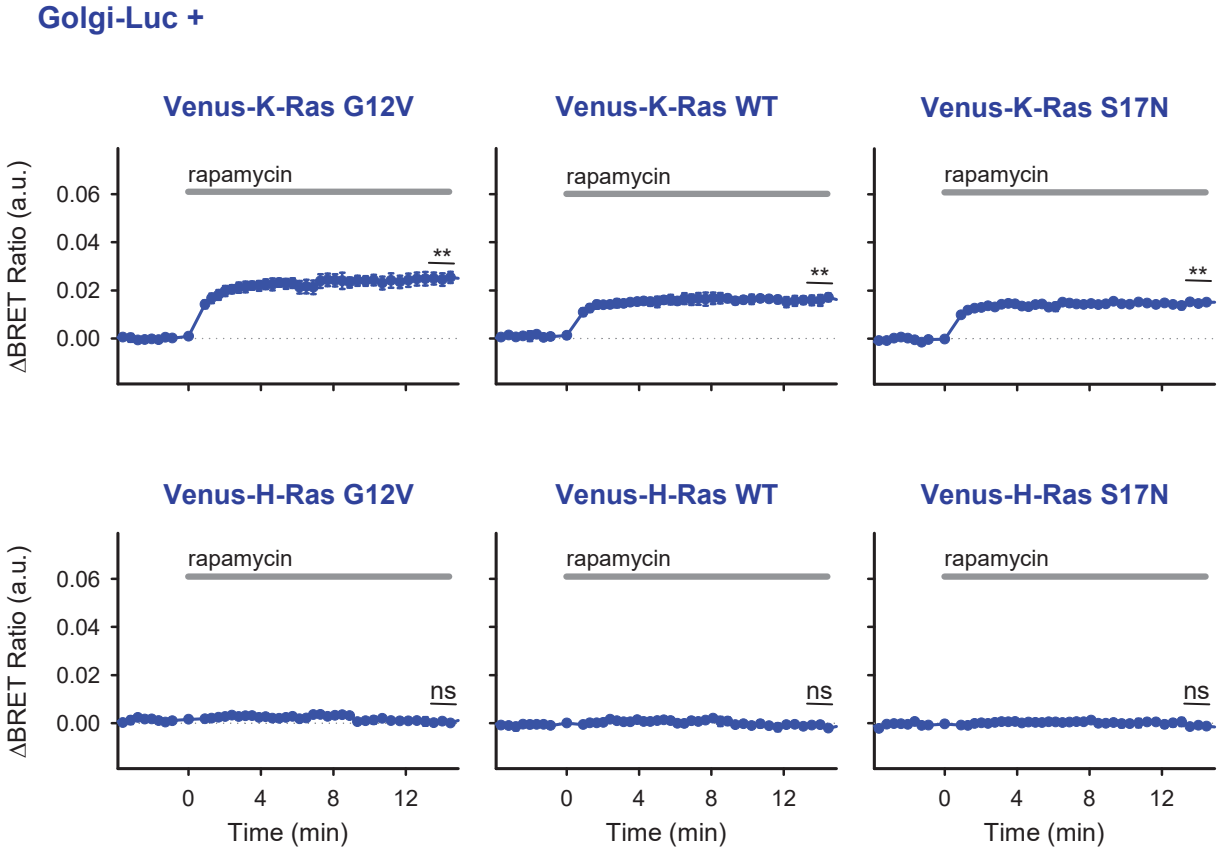
Golgi-Luc +
Venus-K-Ras G12V
Venus-K-Ras WT
Venus-K-Ras S17N
Venus-H-Ras G12V
Venus-H-Ras WT
Venus-H-Ras S17N
rapamycin
ΔBRET Ratio (a.u.)
0.06
0.04
0.02
0.00
**
ns
0
4
8
12
Time (min)

Figure S3