Supplemental Materials

Molecular Biology of the Cell

Shiwarski et al.

Supplemental Figures:

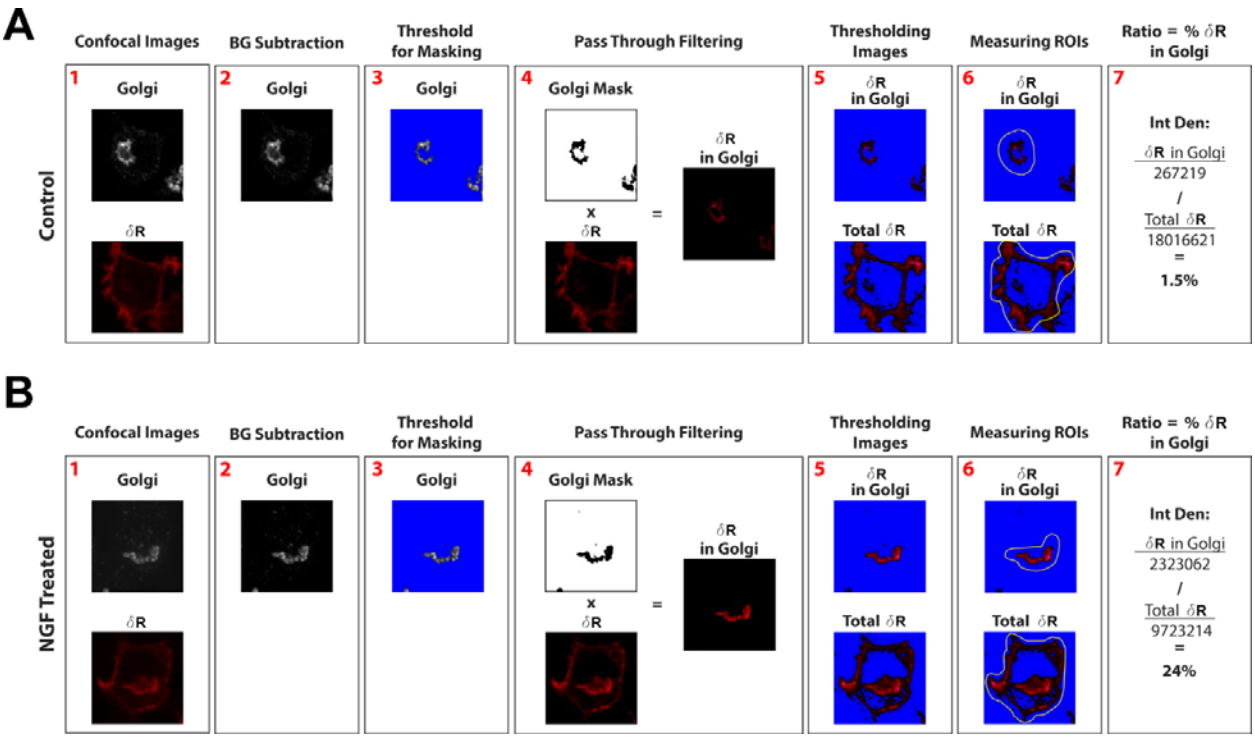

Figure S1: Analysis of δR retention using quantitative fluorescent imaging. A. Depicted is the analysis method we developed for the fixed-cell quantification of the % of δR within the Golgi compared to the total cell fluorescence. 1) Confocal images of fluorescently labeled δR and the Golgi marker (TGN-38) were acquired. 2) The background fluorescence was subtracted from the Golgi channel. 3) The Golgi channel was thresholded to construct a mask of the Golgi region. 4) The Golgi mask was used as a pass-through filter by converting the Golgi mask to a binary image and multiplying it by the δR image. This allows for the measurement of the amount of δR fluorescence within the Golgi region. 5) The δR within the Golgi and total δR images were thresholded to remove bias from background zero pixels. 6) Regions of interest (ROIs) were drawn for the Golgi region and the total δR signal. Measurements were then made in ImageJ to calculate the total fluorescence within these two regions. 7) The fluorescence signal for δR within the Golgi was then divided by the total δR fluorescence signal to get a ratio of the % of δR within the Golgi compared to the total expression of δR. An example is shown for this analysis and calculation for a control treated cell where there is little to no Golgi δR, and an NGF treated cell where there is an apparent Golgi pool of δR.

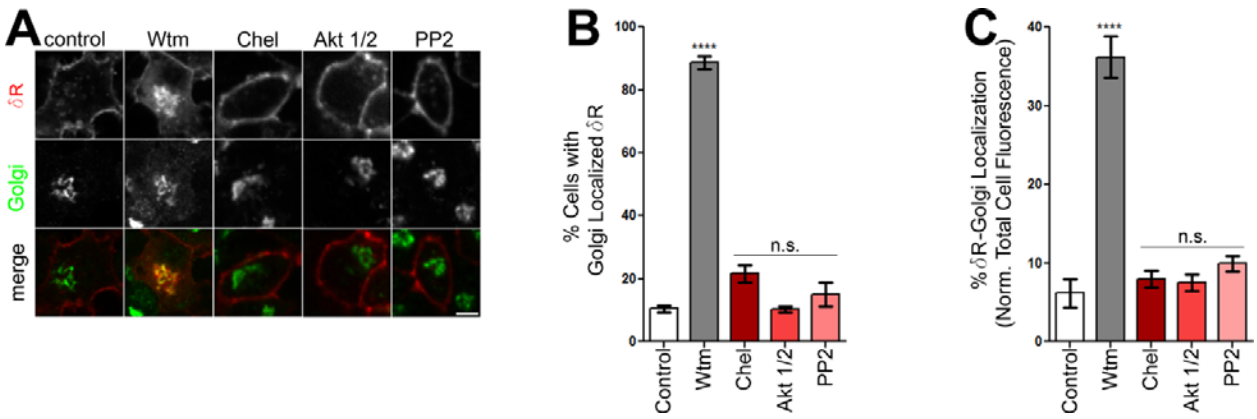

Figure S2: Inhibition of downstream PI3K targets are not sufficient to induce intracellular retention of δR. A. Example images (of *n* = 3 independent experiments) for PC12 cells expressing FLAG-tagged δR treated with inhibitors of PKC by Chelerythrine (Chel, 10 μM), Akt by Akt1/2 Kinase Inhibitor (Akt1/2, 500 nM), and cSrc by PP2 (500 nM). PI3K inhibition by Wortmannin (Wtm, 10 μM) was used as a positive control. **B-C.** Unlike Wtm, inhibition of PI3K downstream targets PKC, Akt, and cSrc do not cause a significant increase in the % of cells with intracellular δR, nor the % of total δR fluorescence localized to the Golgi compared to control (Control, *n*=69 cells; Wtm, *n*=60 cells; Chel, *n*=74 cells; Akt 1/2, *n*=69 cells; PP2, *n*=79 cells; mean ± s.e.m.; ****P<0.0001, n.s. = not significant (P>0.05); by two-sided t-test vs. Control).

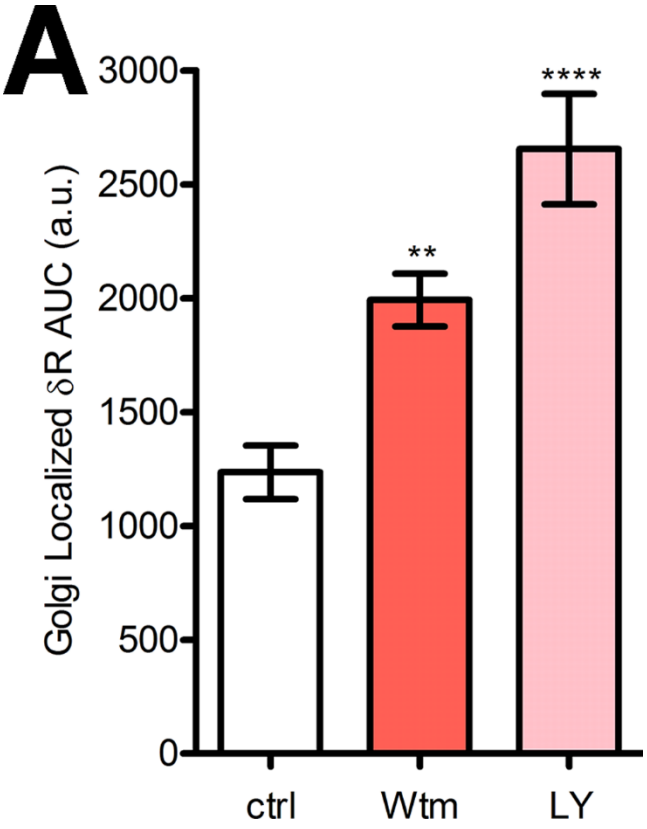

Figure S3: PI3K-inhibition delays the export of δR from the Golgi. A. Area under the curve analysis of the VSVG-δRtail-GFP experiments revealed that PI3K inhibition by Wortmannin (Wtm, 10 μM) and LY294002 (LY, 10 μM) significantly increased the total Golgi-localized signal (control, *n*=12 cells; Wortmannin, *n*=14 cells; LY294002, *n*=12 cells; mean ± s.e.m.; ***P*<0.01; *****P*<0.0001; by two-sided t-test vs. ctrl).

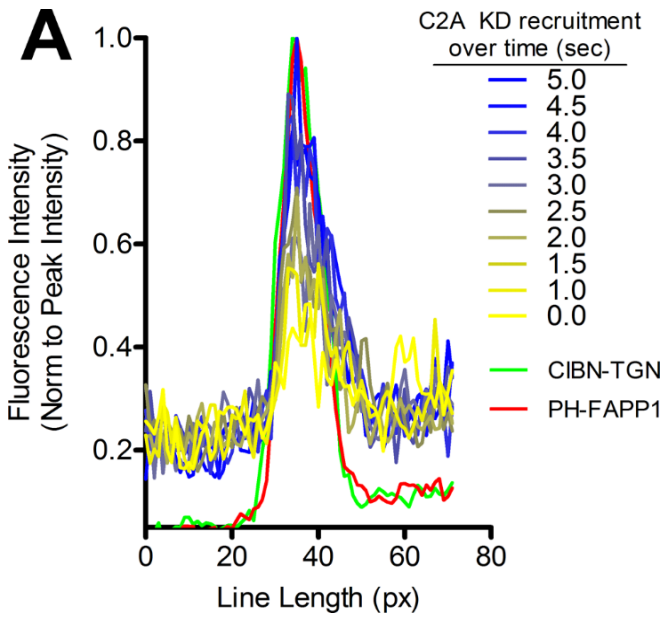

Figure S4: mCherry-CRY2-PI3K-C2A-KD is recruited to the TGN in a time-dependent manner following optogenetic stimulation. A. Over time, the fluorescence intensity profile of the C2A KD along the line scan analysis shows increasing fluorescence intensity (Yellow to Blue), and the peak fluorescence intensity, and the fluorescence intensity distribution for C2A KD (Red), co-align with CIBN-TGN (Green), and PH-FAPP1 (Blue) after 5 seconds of optical stimulation.

Supplemental Table 1:
Table of sequences ordered and 22mer targets

shRNA 22_mer Sequence		Adapter Pairs
#1	GCCACACCATTTCATCCACAAG	5'- TCGAGTGCTGTTGACAGTGAGCG**ACCACACCATTTCATCCACAAG**TAGTGAAGC - 3' 3'-CACGACAACTGTCACTCGC**TGGTGTGGTAAAGTAGGTGTTC**ATCACTTCGGTGT-5' 5'- CACAGATGTA**CTTGTGGATGAAATGGTGTGGC**TGCCTACTGCCTCGGAG - 3' 3'- TACAT**GAACACCTACTTTACCACACCG**ACGGATGACGGAGCCTCTTAA - 5'
#2	GAAGGTTGGCACATACAAGAAT	5'- TCGAGTGCTGTTGACAGTGAGCG**AAAGGTTGGCACATACAAGAAT**TAGTGAAGC - 3' 3'- CACGACAACTGTCACTCGC**TTTCCAACCGTGTATGTTCTTA**ATCACTTCGGTGTC – 5' 5'- CACAGATGTA**ATTCTTGTATGTGCCAACCTTC**TGCCTACTGCCTCGGAG - 3' 3'- TACAT**TAAGAACATACACGGTTGGAAG**ACGGATGACGGAGCCTCTTAA – 5'
#3	AAAGATATTGCTGGATGACAAT	5'- TCGAGTGCTGTTGACAGTGAGCG**CAAGATATTGCTGGATGACAAT**TAGTGAAGC - 3' 3'- CACGACAACTGTCACTCGC**GTTCTATAACGACCTACTGTTA**ATCACTTCGGTGTC – 5' 5'- CACAGATGTA**ATTGTCATCCAGCAATATCTTT**TGCCTACTGCCTCGGAG - 3' 3'- TACAT**TAACAGTAGGTCGTTATAGAAA**ACGGATGACGGAGCCTCTTAA – 5'
#4	TCCAGTCACAGTGCAAAGAAAC	5'- TCGAGTGCTGTTGACAGTGAGCG**CCCAGTCACAGTGCAAAGAAAC**TAGTGAAGC - 3' 3'- CACGACAACTGTCACTCGC**GGGTCAGTGTCACGTTTCTTTG**ATCACTTCGGTGTC – 5' 5'- CACAGATGTA**GTTTCTTTGCACTGTGACTGGA**TGCCTACTGCCTCGGAG - 3' 3'- TACAT**CAAAGAAACGTGACACTGACCT**ACGGATGACGGAGCCTCTTAA – 5'
#5	AGCCTACAACTTGATAAGAAAG	5'- TCGAGTGCTGTTGACAGTGAGCG**CGCCTACAACTTGATAAGAAAG**TAGTGAAGC - 3' 3'- CACGACAACTGTCACTCGC**GCGGATGTTGAACTATTCTTTC**ATCACTTCGGTGTC – 5' 5'- CACAGATGTA**CTTTCTTATCAAGTTGTAGGCT**TGCCTACTGCCTCGGAG - 3' 3'- TACAT**GAAAGAATAGTTCAACATCCGA**ACGGATGACGGAGCCTCTTAA – 5'